

*“Wisdom is the principal thing; therefore get wisdom.
And in all your getting, get UNDERSTANDING”. Proverbs 4:7*

Promoters: Prof. dr. ir. Koen Dewettinck

Ghent University

Department Food Safety and Food Quality

Laboratory of Food Technology and Engineering

Prof. Emmanuel Ohene Afoakwa

University of Ghana

Department of Nutrition and Food Science

Faculty of Science

Dean: Prof. dr. ir. Guido Van Huylenbroeck

Rector: Anne De Paepe

Roger Philip Aidoo

**Functionality of inulin and polydextrose in stevia or
thaumatin sweetened dark chocolate**

Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied
Biological Sciences

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Functionaliteit van inuline en polydextrose in met stevia of thaumatine gezoete donkere chocolade

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Table of contents

TABLE OF CONTENTS	I
LIST OF ABBREVIATIONS.....	IX
SUMMARY	X
SAMENVATTING	XIV
OUTLINE OF THE RESEARCH	1
CHAPTER 1: Introduction to chocolate manufacture	
1.1 Chocolate history and types.....	5
1.2 World consumption of chocolate.....	8
1.3 Chocolate manufacturing processes	12
1.4 Fat (cocoa butter) crystallization during chocolate manufacture	14
1.5 Quality parameters in chocolate manufacture	17
1.5.1 Flow properties.....	17
1.5.2 Particle size distribution	20
1.5.3 Texture and Appearance	22
1.5.4 Microstructure.....	23
1.5.5 Moisture	24
CHAPTER 2: Industrial manufacture of sugar-free chocolates – Applicability of alternative sweeteners and carbohydrate polymers as raw materials in product development – a review	
2.1 Introduction.....	27
2.2 Functionality of sucrose in chocolate manufacture.....	28
2.3 Alternative sweetening solutions in chocolate manufacture	29
2.3.1 High potency sweeteners	29
2.3.1.1 Stevia rebaudioside A	29
2.3.1.2 Thaumatin.....	35

2.3.2 Bulk sweeteners	38
2.3.2.1 Polyols (sugar alcohols)	38
2.3.2.2 Tagatose.....	40
2.3.2.3 Trehalose	42
2.3.2.4 Isomaltulose.....	44
2.3.3 Low-digestible carbohydrate polymers.....	46
2.3.3.1 Polydextrose	46
2.3.3.2 Inulin and oligofructose.....	49
2.3.3.3 Maltodextrin	51
2.3.4 Laxation and low – digestible carbohydrate polymers	53
2.3.5 Applicability and suitability of different sweeteners and polymers in chocolate processing.....	53
2.3.5.1 Polyols.....	54
2.3.5.2 Intense sweeteners	56
2.3.5.3 Carbohydrate polymers	56
 CHAPTER 3: Optimization of processing conditions and rheological properties using Stephan mixer as conche in small-scale chocolate processing	
3.1 Introduction.....	62
3.2 Research strategy	63
3.3 Materials and methods	64
3.3.1 Raw materials	64
3.3.2 Sample preparation using the Stephan mixer.....	64
3.3.3 Sample preparation using Buhler ELK’Oline Conche	65
3.3.4 Analytical methods	67
3.3.5 Statistical analysis.....	67
3.4 Results and discussion.....	68

3.4.1 Effect of conching time and temperature	68
3.4.2 Effect of blade rotary speed	72
3.4.2.1 Constant rotary speed for dry and wet conching.....	72
3.4.2.2 Variable rotary speed at constant dry conching speed.....	74
3.5 Conclusions.....	76
 CHAPTER 4: Optimization of inulin and polydextrose mixtures as sucrose replacers during sugar-free chocolate manufacture – Rheological, microstructure and physical quality characteristics	
4.1 Introduction.....	78
4.2 Research strategy	79
4.3 Materials and methods	79
4.3.1 Raw materials	79
4.3.2 Experimental design and sample preparation	79
4.3.3 Tempering procedure.....	80
4.4 Analytical methods.....	81
4.4.1 Rheological properties.....	81
4.4.2 Moisture	81
4.4.3 Particle size distribution (PSD)	81
4.4.4 Hardness	82
4.4.5 Colour	82
4.4.6 Microscopy	82
4.5 Data analysis.....	83
4.6 Results and discussion.....	83
4.6.1 Rheological properties.....	83
4.6.1.1 Casson plastic viscosity	83
4.6.1.2 Casson yield stress	85

4.6.2 Microscopy	88
4.6.3 Colour	90
4.6.4 Hardness	91
4.6.5 Moisture	92
4.7 Conclusions.....	93
 CHAPTER 5: Impact of particle size distribution (PSD) and fat content on rheological, mechanical and melting properties of sugar-free dark chocolates processed using inulin and polydextrose mixtures as bulking agents	
5.1 Introduction.....	95
5.2 Research strategy	96
5.3 Materials and methods	96
5.3.1 Raw materials	96
5.3.2 Chocolate production	96
5.3.3 Tempering procedure	97
5.4 Analytical methods.....	97
5.4.1 Particle size analysis	97
5.4.2 Rheological properties.....	98
5.4.3 Hardness	98
5.4.4 Melting properties.....	98
5.5 Statistical analysis.....	98
5.6 Results and discussion	98
5.6.1 Rheological properties.....	99
5.6.1.1 Casson viscosity	99
5.6.1.2 Casson yield stress	103
5.6.2 Hardness	105
5.6.3 Melting properties.....	106

5.7 Conclusions.....	109
CHAPTER 6: Functionality of inulin and polydextrose mixtures in defining the	
microstructural behaviour and physical quality characteristics of sugar-free	
dark chocolate	
6.1 Introduction.....	111
6.2 Research strategy	112
6.3 Materials and methods	112
6.3.1 Raw materials	112
6.3.2 Experimental design	112
6.3.3 Tempering procedure.....	113
6.4 Analytical methods.....	113
6.4.1 Density.....	113
6.4.2 Moisture (bulking agents)	113
6.4.3 Microscopy (bulking agents)	114
6.4.4 X-ray diffraction analysis	114
6.4.5 Melting properties (bulking agents).....	114
6.4.6 Rheological properties.....	115
6.4.7 Texture measurements	115
6.4.8 Colour	115
6.4.9 Moisture (chocolate)	115
6.4.10 Melting properties (chocolate).....	115
6.4.11 Microscopy (chocolate)	115
6.5 Statistical analysis.....	115
6.6 Results and discussion.....	116
6.6.1 Characterization of bulking agents.....	116
6.6.1.1 Physicochemical properties of bulking agents	116

6.6.1.2 Microscopic properties of bulking agents	117
6.6.1.3 Thermal behaviour of bulking agents.....	119
6.6.2 Functionality of inulin and polydextrose on physicochemical properties of sugar-free dark chocolates	121
6.6.2.1 Rheological properties.....	121
6.6.2.1.1 Casson viscosity	122
6.6.2.1.2 Casson yield stress	123
6.6.2.2 Texture.....	125
6.6.2.3 Moisture	126
6.6.2.4 Colour.....	127
6.6.2.5 Melting properties.....	129
6.6.3 Process optimization for sugar-free dark chocolate manufacture	130
6.6.3.1 Rheological properties.....	130
6.6.3.1.1 Casson viscosity	131
6.6.3.1.2 Casson yield stress	132
6.6.3.2 Texture.....	133
6.6.3.3 Moisture	134
6.6.3.4 Colour.....	135
6.6.3.5 Melting properties.....	135
6.7 Conclusions.....	136
CHAPTER 7: Equivalence sweetening and bitterness sensations evaluated by time-intensity measurements on different stevia rebaudioside A sweeteners and thaumatin as sucrose replacers in sugar-free chocolate manufacture	
7.1 Introduction.....	138
7.2 Research strategy	140
7.3 Materials and methods	140

7.3.1 Raw materials	140
7.3.2 Panel screening and training	140
7.3.3 Sample preparation	141
7.3.4 Sensory procedures	141
7.3.4.1 Maximum sweetness intensity (I _{max} stat)	142
7.3.4.2 Bitterness level	143
7.3.4.3 Time-intensity analysis	143
7.3.5 Data analysis	144
7.4 Results and discussion	145
7.4.1 Stevia rebaudioside A	145
7.4.1.1 Sweetness profile	145
7.4.1.2 Bitterness levels	147
7.4.2 Thaumatococcus	151
7.4.2.1 Sweetness profile	151
7.5 Conclusions	152
CHAPTER 8: Rheological, physical and sensory characteristics of sugar-free chocolates	
processed using inulin/polydextrose mixtures and sweetened with stevia and	
thaumatococcus extracts	
8.1 Introduction	155
8.2 Research strategy	156
8.3 Materials and methods	157
8.3.1 Raw materials	157
8.3.2 Chocolate production	157
8.3.3 Tempering procedure	157
8.4 Analytical methods	157
8.4.1 Rheological properties	157

8.4.2 Moisture	158
8.4.3 Hardness	158
8.4.4 Colour	158
8.4.5 Melting properties.....	158
8.5 Sensory analysis.....	158
8.6 Data analysis.....	159
8.7 Results and discussion.....	159
8.7.1 Rheological properties.....	159
8.7.2 Moisture	162
8.7.3 Hardness	163
8.7.4 Colour	163
8.7.5 Melting properties.....	165
8.7.6 Consumer tests.....	167
8.8 Conclusions.....	171
 CHAPTER 9: General conclusions and recommendations for further studies	
REFERENCES	176
CURRICULUM VITAE	206
ACKNOWLEDGEMENTS.....	208

ABBREVIATIONS USED

ADI	Acceptable Daily Intake
ANOVA	Analysis of Variance
CTU	Chocolate Temper Units
DP	Degree of Polymerization
DSC	Differential Scanning Calorimetry
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization
FCC	Food Chemicals Codex
GRAS	Generally Recognized As Safe
HPS	High Potency Sweeteners
ICA	International Confectionery Association
ICCO	International Cocoa Organization
IOCCC	International Office of Cocoa, Chocolate and Confectionery
JECFA	Joint Expert Committee on Food Additives
LDC	Low Digestible Carbohydrate
PS	Particle Size
PSD	Particle Size Distribution
PSI	Particle Size Interval
SEM	Scanning Electronic Microscopy
T-I	Time Intensity
USFDA	United States Food and Drugs Administration
WHO	World Health Organization

SUMMARY

Dark chocolate is a dense fat-based suspension of solid particles comprising 60-70% sugar and cocoa solids. Sucrose is the conventional sweetening agent prevailing in the traditional chocolate processing industry due to the multi-functional properties of sweetness, bulkiness and textural characteristics it offers to products. Today's consumers are concerned about the high sugar levels, calories and cariogenicity effects in confectionery products, hence growing the popularity of 'light' and 'sugar-free' products. These products offer varied levels of quality and taste dissimilar to their sugar counterparts. While the use of sucrose prevails in traditional food industry, numerous nutritive and non-nutritive sweeteners offer new opportunities for the chocolate manufacturer. Given that all the sugar needs to be replaced, it is most challenging to develop sugar-free chocolates. The difficulty is in finding a replacer for sucrose that will provide the desired taste, bulk, texture and appearance. The primary aim of this research was to investigate the functionality of inulin and polydextrose as sucrose replacers in sugar-free dark chocolates sweetened with stevia or thaumatin.

Conching is an important stage in chocolate processing. During this stage, a heterogeneous, flaky, dry refined chocolate paste is turned into a free-flowing suspension of molten chocolate combined with the removal of moisture, acids and undesirable flavours, and consequently leading to the development of the final chocolate texture and flavour. Available conches require raw materials between 4 kg (laboratory scale) to multiple tonnes (industrial scale) and the conching process lasts for as little as 4 to 6 hours to a few days (up to 3). The conching process thus results in high raw material usage and causes delays in processing times even when small quantities of finished chocolate are required for chocolate-based production applications such as panning, enrobing and extrusion. In this work, the Stephan mixer was optimized for use as a conche in small-scale chocolate production. Although not all aspects of the chocolate conching process were considered, dark chocolate dry conched at 65 °C for 10 min with 1500 rpm blade rotational speed followed by wet conching at 50 °C for 15 min with blade rotational speed of 1500 rpm, resulted in similar flow properties as samples conched for 6 h in a Bühler Elk'Olino conche (~4kg capacity). This offers opportunities for down-scale processing at reduced conching times with assurances in product quality.

Targeting sugar replacement in chocolate requires a clear understanding of the functionality of bulking agents in a chocolate matrix as well as the sweetness brought by intense sweeteners. This work investigated the influences of inulin and polydextrose mixtures as bulk ingredients on sugar-free dark chocolate microstructure and physical quality characteristics. Increasing inulin concentration with simultaneous reduction in polydextrose content resulted in an increase in Casson viscosity with the highest Casson viscosity recorded for formulations with 100% inulin. In contrast, a general trend emerged where decreasing polydextrose concentration with simultaneous increase in inulin concentration led to a decrease in the Casson yield stress. This was as a result of the particle size distribution, with chocolate formulations containing high levels of polydextrose exhibiting lower particle sizes than the other formulations. A mixture of 25% inulin and 75% polydextrose was established as the optimum blend for the development of sugar-free dark chocolate. Formulations with the optimum blend recorded no significant ($P>0.05$) differences in Casson yield stress, Casson viscosity and D_{90} values compared to the reference sample made with sucrose.

Chocolate quality attributes are highly dependent on inherent size distribution of solid particles from sugar, milk and cocoa solids, fat phase composition and presence of emulsifiers. The effects of particle size distribution and fat content on the rheological, textural, melting properties and appearance of the sugar-free dark chocolate produced with a polydextrose/inulin mixture as bulking agent were investigated. Both particle size and fat content had significant ($P\leq 0.05$) effects on chocolate viscosity. The effect of particle size on Casson viscosity and yield stress was more pronounced at lower fat contents. The Casson yield stress increased from 17.6 Pa to 23.2 Pa with increase in particle size from 20 μm to 50 μm at the 27% fat level. Hardness was noted to decrease linearly with increasing fat content. Significant ($P\leq 0.05$) increases in hardness with increase in particle size were also noted at 27% fat level. Samples refined to particle sizes of 20 μm and 30 μm were however not significantly ($P>0.05$) different for hardness at 30% fat. Particle size however had a very small influence on chocolate melting properties.

To gain further insights into the functionality of inulin and polydextrose in sugar-free dark chocolate, the physico-chemical properties of the bulking agents were studied. Hereto their thermal behaviour, glass transition temperature range, moisture content and density were compared to that of sucrose. Inulin recorded a lower density than polydextrose which could

account for the different trends observed in chocolate flow properties. Sucrose replacement by the bulking agents was done on weight basis therefore chocolates with high levels of inulin will have more solids per volume and increased particle volume fraction and solids' surface area. This might have a major impact on particle collision and aggregation in a colloidal dispersion such as chocolate. The moisture content of Inulin and polydextrose were significantly ($P < 0.05$) higher than that of sucrose indicating the hygroscopic nature of the sugar replacers. In chocolate, moisture aggregates sugar particles to form gritty lumps and moisture at sugar particle surfaces increases friction between the particles, resulting in increase in apparent viscosity. Scanning electron microscopy (SEM) suggested that polydextrose is amorphous where as visualisations of inulin gave an indication of an amorphous material with crystalline regions. The amorphous nature of polydextrose and inulin were confirmed by X-ray diffraction analysis which showed no distinct peaks in the diffraction pattern of both bulking agents. The thermal transition of inulin as measured by differential scanning calorimetry (DSC) however showed a shallow and broad peak confirming the presence of crystalline regions.

Further optimizations regarding formulation and processing conditions were conducted by varying lecithin concentration and wet conching time. Increasing lecithin concentration from 0.4% to 0.6% promoted adequate adsorption of the emulsifier around solid particle surfaces, thereby lowering the interfacial tension between the dispersed and the continuous phase leading to a significant reduction in the Casson viscosity. Increasing wet conching time from 30 to 60 min also enhanced the effective coating of solid particles with cocoa butter, thus further lowering the plastic viscosity.

As previously mentioned, sugar replacement in chocolate requires a clear understanding of both the physicochemical properties of the bulk replacers as well as the sweetness brought by intense sweeteners. Stevia rebaudioside A and thaumatin are considered natural sweeteners and possess varying sweetening potentials and bitter aftertastes. This part of the research compared the sweetness profiles of stevia rebaudioside A and thaumatin, first in a water matrix and then finally in a chocolate matrix with a polydextrose/inulin mixture as bulking agent. The sweetness profile of aqueous solution of thaumatin showed a slow sweetness onset which increased to a maximum after 10 s. The sweetness then declined

gradually and levelled off after 50 s. Aqueous solution of stevia rebaudioside A however had a fast onset of sweetness with consistent decrease in sweetness intensity within the entire evaluation time. Sensory evaluation of chocolates developed with the intense sweeteners showed much preference for the stevia sweetened chocolate than the thaumatin sweetened chocolate. Chocolate sweetened with stevia was rated much sweeter and better tasting than chocolate sweetened with thaumatin. In contrast, less perceived sweetness was mentioned as the greatest deficiency of the thaumatin sweetened chocolate.

The suitability and applicability of different sweeteners and biopolymers in chocolate processing have been reviewed. To the best of our knowledge, this research is the first to report results of the application of thaumatin as a sweetener in sugar-free dark chocolate manufacture. This research also reports for the first time, results of a comparative study of the intense sweeteners (stevia and thaumatin) in sugar-free dark chocolates with inulin/polydextrose mixtures as bulking agents. Findings from this research will make significant contributions to chocolate and confectionery science by providing in-depth understanding of the microstructural behaviour of inulin and polydextrose in sugar-free dark chocolate manufacture. The health benefits that can be derived from consumption of the developed sugar-free dark chocolate cannot be overemphasized. Not only the dietary fibre properties of the bulking agents are important but also the prebiotic properties of inulin, resulting in better health and reduction in the risk of many diseases.

SAMENVATTING

Donkere chocolade is een vetgebaseerde suspensie met daarin 60-70% vaste deeltjes, meer bepaald suiker en cacaopartikels. Klassiek wordt sucrose als zoetstof gebruikt vanwege de multifunctionele eigenschappen (zoetheid, bulk en textuur) ze biedt aan chocolade. Vandaag de dag zijn de consumenten verontrust over de hoge suikergehaltes en calorieën in zoetwaren en hun effecten op tandbederf. Vandaar dat er een stijgende vraag is naar hoog-kwalitatieve *light* en suikervrije producten. Tegenwoordig zijn er dergelijke producten variërend in kwaliteit en met afwijkende smaak tegenover de referentie met sucrose op de markt. Desalniettemin het gebruik van sucrose overheerst in de traditionele voedingsindustrie, biedt de beschikbaarheid van talrijke suikervervangers nieuwe opportuniteiten voor de chocoladeproducent. De uitdaging ligt in het feit een vervanger te vinden die het product de gewenste smaak, bulk, textuur en uitzicht verschaft. Het hoofddoel van dit onderzoek was dan ook om de functionaliteit van de bulkstoffen inuline en polydextrose in suikervrije donkere chocolade gezoet met stevia of thaumatococcus te onderzoeken.

Concheren is een belangrijke stap in het chocolade productieproces. Tijdens deze stap verandert het heterogene, vlokkerige, droge gewalst product in een vloeibare chocolade en worden vocht, vluchtige zuren en ongewenste aroma's verwijderd. Dus hier ontwikkelt zich de finale textuur en smaak van chocolade. Beschikbare conches behoeven grondstoffen vanaf 4 kg (laboschaal) tot enkele tonnen (industriële schaal) en tijden tussen 4 uur en 3 dagen. Het concheerproces verbruikt dus een grote massa ingrediënten en veroorzaakt een vertraging in het productieproces, zelfs al zijn er slechts kleine hoeveelheden chocolade nodig voor specifieke toepassingen zoals pannen, enroberen en extrusie. In dit onderzoek werd het gebruik van de Stephan menger geoptimaliseerd als alternatief voor concheren op kleine schaal. Hoewel niet alle aspecten van het concheerproces werden belicht, resulteerde een "droge" concheerstap bij 65°C voor 10 min aan een rotatiesnelheid van 1500 rpm gevolgd door een "natte" concheerstap bij 50°C voor 15 min aan dezelfde rotatiesnelheid in gelijkaardige vloeiparameters dan met een conche op laboschaal (Bühler Elk'Oline, 4 kg). Dit biedt mogelijkheden voor de Stephan menger om chocolade te produceren op kleine schaal in een kleiner tijdsbestek met behoud van productkwaliteit.

Suikervervanging in chocolade vereist duidelijke inzichten in zowel de functionaliteit van bulkstoffen in een chocoladematrix als de zoetheid van intensieve zoetstoffen. Hier werden de invloeden van inuline en polydextrose mengsels als bulkingrediënten op de microstructuur en fysische kwaliteitsattributen van suikervrije donkere chocolade bestudeerd. Een stijging in de inulineconcentratie en simultane daling van het polydextrosegehalte resulteerde in een stijging van de Casson viscositeit. De hoogste viscositeit werd dan ook geobserveerd in de formulering met 100% inuline. De omgekeerde trend bleek waar voor de Casson vloeigrens. Dit gedrag kon worden toegewezen aan de partikelgroottedistributie, waarbij hogere concentraties polydextrose resulteerden in lagere partikelgroottes. Een mengsel inuline/polydextrose 25/75 bleek het meest beloftevol voor de ontwikkeling van suikervrije donkere chocolade. De chocolade met dit mengsel vertoonde geen significante verschillen ($P > 0.05$) in Casson vloeigrens, Casson viscositeit en D_{90} met de referentie gezoet met sucrose.

De kwaliteitsaspecten van chocolade zijn in hoge mate afhankelijk van de inherente groottedistributie van de vaste deeltjes (suiker, melkpoeder en cacaobestanddelen), vetfase en de aanwezigheid van emulgatoren. De effecten van de partikelgroottedistributie en vetgehalte op het reologisch gedrag, de textuur, smelteigenschappen en uitzicht van suikervrije donkere chocolade geformuleerd met inuline/polydextrose mengsels werden onderzocht. Zowel de partikelgrootte als vetgehalte had een impact ($P \leq 0.05$) op de plastische viscositeit. Het effect van de partikelgrootte op de vloeiparameters bleek wel meer uitgesproken bij lagere vetgehaltes. De Casson vloeigrens nam toe van 17.6 Pa tot 23.2 Pa wanneer de partikelgrootte steeg van 20 μm tot 50 μm bij een vetgehalte van 27%. De hardheid daalde lineair met toenemend vetgehalte. Daarenboven werd een significante stijging ($P \leq 0.05$) in hardheid vastgesteld bij hogere partikelgroottes en een vetgehalte van 27%. Chocolades met een vetgehalte van 30% en gewalst tot partikelgroottes van 20 μm en 30 μm waren niet significant verschillen ($P > 0.05$) betreffende hardheid. Verder bleek de partikelgrootte een zeer kleine invloed te hebben om de smelteigenschappen.

Om verdere inzichten in de functionaliteit van inuline en polydextrose in suikervrije donkere chocolade te vergaren, werden de fysicochemische eigenschappen (thermisch gedrag, glastransitietemperatuur, vochtgehalte en densiteit) van de bulkstoffen bepaald en vergeleken met die van sucrose. Inuline vertoonde een lagere densiteit dan polydextrose

wat mogelijks hun effect op de vloeikarakteristieken van chocolade verklaart. Suikervervanging door bulkstoffen gebeurde op gewichtsbasis zodat het gebruik van inuline leidde tot een hogere partikelvolumefractie en specifiek oppervlak. Dit kan een impact hebben op interacties en aggregatie tussen de vaste deeltjes in een colloïdale dispersie zoals chocolade. Het vochtgehalte van inuline en polydextrose was significant ($P \leq 0.05$) hoger dan dat van sucrose wat te wijten is aan hun hygroscopiciteit. Vocht kan zorgen voor aggregatie van suikerpartikels in chocolade wat leidt tot zanderigheid en een verhoogde wrijving tussen de vaste deeltjes in beweging. Laatstgenoemd fenomeen resulteert in een hogere schijnbare viscositeit. Visualisaties via scanning elektronenmicroscopie (SEM) suggereerden dat polydextrose en inuline (deels) amorf zijn. De amorfe natuur van deze bulkstoffen werd bevestigd via X-stralenanalyse die geen duidelijke diffractiepieken vertoonde. Het thermische profiel van inuline, gemeten met differentiële scanning calorimetrie (DSC), vertoonde een brede endotherme piek wat de aanwezigheid van kristallijne regio's aantoonde.

Een doorgedreven optimalisatie van de formulering van suikervrije donkere chocolade en de procescondities werd uitgevoerd door variatie van het lecithinegehalte en “natte” concheertijd. Een stijgende lecithineconcentratie van 0.4% tot 0.6% bevorderde de adsorptie van de emulgator op het oppervlak van de deeltjes, waardoor de grensvlakspanning tussen de gedispergeerde en continue fase daalde met een significante daling van de Casson viscositeit tot gevolg. Bovendien verhoogde een “natte” concheertijd van 60 min t.o.v. 30 min de effectieve *coating* van de vaste deeltjes met cacaoboter, met opnieuw een verlaging van de plastische viscositeit tot gevolg.

Zoals reeds vermeld, zijn inzichten in zowel de fysicochemische eigenschappen van de bulkstoffen als de zoetheid van de intensieve zoetstoffen van belang bij de ontwikkeling van suikervrije chocolade. Stevia rebaudioside A en thaumatine worden aanzien als natuurlijke zoetstoffen en vertonen een verschillend potentieel in zoetkracht en bittere nasmaken. In dit deel van het onderzoek werden de zoetheidsprofielen in water en suikervrije chocolade (inuline/polydextrose mengsel als bulkstof) van beide intensieve zoetstoffen vergeleken. Het profiel van een waterige thaumatine oplossing vertoonde een trage start van zoetheid die

steeg tot een maximum na 10 seconden. De zoetheid vlakke af na 50 seconden. De waterige oplossing van Stevia rebaudioside A echter had een snellere start in zoetheid met een consistente daling binnen de tijd van sensorische evaluatie. Het sensorisch onderzoek toonde aan dat er een duidelijke voorkeur was voor suikervrije donkere chocolade gezoet met stevia. Deze intensieve zoetstof zorgde ervoor dat de chocolade als zoeter en beter werd ervaren dan de chocolade met thaumatine. De lagere zoetheid van de suikervrije chocolade gezoet met thaumatine werd dan ook als het grootste gebrek beoordeeld.

Er werd teruggeblikt naar de geschiktheid en toepasbaarheid van verschillende zoetstoffen en biopolymeren in de verwerking van chocolade. Zover we weten is dit onderzoek het eerste dat wetenschappelijke resultaten van de toepasbaarheid van thaumatine als intensieve zoetstof in suikervrije donkere chocolade rapporteert. Bovendien is dit onderzoek ook het eerste dat intensieve zoetstoffen, m.n. stevia en thaumatine, met inuline/polydextrose mengsels als bulkstoffen in de chocoladematrix vergelijkt. Bevindingen van deze studie zullen substantiële bijdragen leveren tot het wetenschappelijk onderzoek rond chocolade en zoetwaren. We denken hierbij aan de inzichten in het microstructureel gedrag van inuline en polydextrose in de productie van suikervrije donkere chocolade. De voordelen voor de gezondheid bij de consumptie van de ontwikkelde suikervrije donkere chocolade kunnen niet genoeg benadrukt worden. Niet alleen het feit dat de bulkstoffen voedingsvezels zijn, maar ook de prebiotische eigenschappen van inuline die resulteren in een betere gezondheid en een verminderd risico of vele ziekten zijn hierbij belangrijk.

OUTLINE OF THE RESEARCH

Chocolate is one of the fastest growing products within the confectionery industry. During processing, the components are mixed, refined and conched to attain desired rheological properties and physical characteristics. Sucrose is utilized up to 30-60% in chocolate and this confers multiple functional properties on chocolate including sweetness, bulkiness and mouthfeel (texture). Its impact on rheological properties is also important for the end product quality. The high sugar content of chocolate has however led to the search for low calorie, low glycaemic index, healthier alternatives. Polydextrose and inulin are considered fibers that do not only increase the bulkiness of food and its rapid movement through the gastrointestinal system, but also help in preventing constipation and possible colon and rectal cancer. Stevia rebaudiana and thaumatin are natural intense sweeteners with sensory properties superior to those of many high-potency sweeteners. The overall strategic objective of this doctoral research was to investigate the functionality of inulin and polydextrose as sucrose replacers in sugar-free dark chocolates with stevia or thaumatin as intense sweeteners. **Figure 1** schematically summarizes the outline of this research.

The first two chapters give extensive literature on chocolates and their sugar-free counterparts. **Chapter 1** introduces the different types of chocolates, chocolate manufacturing processes and key quality parameters in chocolate production. **Chapter 2** characterizes the major alternative sweetener types and bulking agents relevant to the chocolate industry. The applicability and suitability of the different sweeteners and carbohydrate polymers in sugar-free chocolate production are critically reviewed. To reduce raw material wastage during the conching process, the suitability of the Stephan mixer for use as an alternative conche is investigated in **Chapter 3**. The laboratory Bühler Elk'Oline conche requires at least 4kg refined chocolate mix and 6 h processing time for effective operation so the objective was to reduce cost and processing time. The Stephan mixer proved to be suitable for conching small-scale (approximately 1 kg) chocolate productions when considering chocolate flow properties and could be explored as a fast and cost effective conching method.

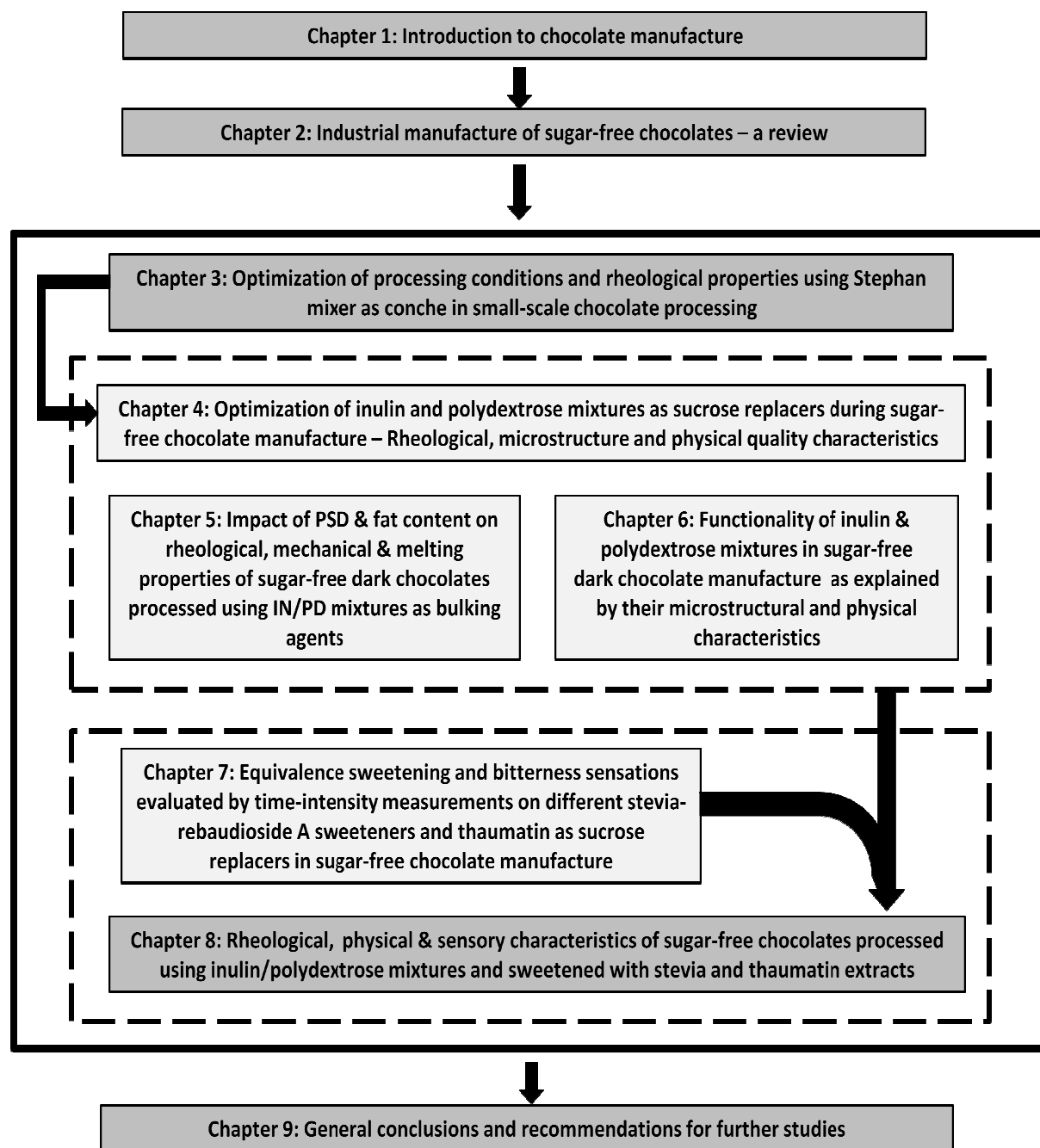


Figure 1. Outline of the research

Aiming for sugar replacement in chocolate requires a clear understanding of the bulk role sugar fulfils in the chocolate matrix and research to take over this bulk role by bulk sweeteners. **Chapters 4, 5 and 6** discuss results of the effects of inulin and polydextrose mixtures as sucrose replacers on quality characteristics of sugar-free dark chocolate. Optimum concentrations of the bulking agents are investigated in **chapter 4** whereas **chapters 5 and 6** discuss results of the effects of composition (bulking agents, fat and

lecithin), particle size (PS) and processing conditions on the textural, melting behaviour and physical properties of the developed sugar-free dark chocolates.

In the end, the sweetness provided by the intense sweeteners stevia and thaumatin is evaluated, firstly outside a chocolate matrix in **Chapter 7**, and then finally in a chocolate matrix (with inulin and polydextrose mixture as bulking agents) in **Chapter 8**. **Chapter 9** gives general conclusions of this research and makes recommendations for further studies.

Chapter 1: Introduction to chocolate manufacture

1.1 Chocolate history and types

Chocolate is the main product developed from cocoa beans. Cocoa beans are derived from the fruit pods of the cocoa tree (*Theobroma cacao* L.) which is cultivated in plantations in the equatorial zone with the Ivory Coast, Brazil, and Ghana as the major producers (Ntiamoah, 2008). About 71% of the cocoa consumed around the world comes from Africa, especially from Ivory Coast, Ghana and Nigeria. Cocoa beans are made up of three main parts namely, the testa (seed coat), the embryo and the cotyledon (Thompson et al., 2001; Afoakwa, 2010). Attached to the testa is the sugary, white mucilaginous pulp which is formed during pod development from an endocarp meristem (Biehl and Ziegleder, 2003). The physical and chemical properties of cocoa beans are very complex and change throughout the life of the bean, mainly depending on the processing it receives and on geographical origin (Bertazzo et al., 2012). Table 1.1 presents the composition of West African (*Forastero*) cocoa beans.

Table 1.1 Chemical composition of unfermented West African (*Forastero*) cocoa beans
(Rohan, 1963 ; Reineccius et al., 1972 ; Dand, 1999)

Constituents	Dried beans (%)
Water	3.5 - 3.7
Fat	52.1 – 57.0
Ash	2.6 - 3.2
Nitrogen	
Total nitrogen	2.3 - 2.5
Theobromine	1.4 - 1.7
Caffeine	0.1 - 0.7
Starch	6.1 - 7.0
Crude fibre	2.1 - 3.2

Reineccius et al. (1972) reported fresh unfermented cocoa beans contained 15.8 mg/g sucrose and trace amounts of fructose, sorbose, mannitol and inositol. Berbert (1979) suggested sucrose content at 24.8 mg/g unfermented beans formed about 90% of total sugars (27.1 mg/g). The reducing sugars, fructose and glucose form about 6% (0.9 and 0.7 mg/g, respectively) and others (including mannitol and inositol) at <0.50 mg/g. Differences have been attributed to method and time of harvesting, type and origin of cocoa beans

(Reineccius et al., 1972). Cocoa is also rich in polyphenols, specifically catechins (flavan-3-ols) and procyanidins, stored in cotyledon pigment cells and cocoa leaves (Osman et al., 2004). Three groups of polyphenols can be differentiated: catechins or flavan-3-ols (ca. 37%), anthocyanins (ca. 4%) and proanthocyanidins (ca. 58%). Nazaruddin et al. (2001) reported total polyphenols ranged from 45–52 mg/g in cocoa liquor, 34–60 in beans and 20–62 in powder. Cocoa beans contain stimulant substances, such as theobromine, caffeine, and theophylline, named purinic alkaloids (Bertazzo et al., 2012).

Raw cocoa beans have an astringent flavour and have to be fermented, dried, and roasted to obtain the desired characteristic cocoa flavour. Dried fermented beans are transported under controlled storage conditions to major chocolate manufacturing countries or processed in the origin country to add value with requirements for traceability in quality assurance (Cleenwerck, 2007). The final chocolate flavor is influenced by the origin and cultivar of the cocoa beans, the on-the-farm fermentation and drying process, and the roasting and further processing performed by the cocoa and chocolate manufacturer (Danquah, 2003). For example, pod storage and duration of fermentation will affect pH and temperature during fermentation thus influencing enzyme activities and flavour development and hence acidity, bitterness and astringency of the processed cocoa beans (Hansen et al., 1998).

For centuries cocoa-rich chocolate has been known not only for its good taste but also for its proposed health effects. Indeed, the Incas considered it the drink of gods, an association that gave rise to the scientific name of the cocoa tree, *Theobroma cacao*, from the Greek words *theo* (god) and *broma* (drink). The first hints of cocoa consumption date back to 1600 BC. In Honduras, archeologists uncovered elaborately designed bowls of this period that are believed to have been used by the Aztecs to drink liquid cocoa thousands of years ago (Henderson et al., 2007). Aztec Emperor Montezuma was a keen admirer of cocoa calling it a “divine drink” which builds up resistance and fights fatigue. A cup of this precious drink permits a man to walk for a whole day without food. In the language of the Aztecs, this drink was called *chocolatl* (Dillinger et al., 2000).

According to Minifie (1989) until the early 1800s the only product was a very fatty chocolate drink prepared from the whole cocoa beans, sugar and spices. Since then production and

consumption of chocolate products have increased predominantly in the Western world and chocolate's role as a symbol of pleasure has remained intact in this area of the world and has contributed greatly to their economy (Anon, 1993). Van Houten developed cocoa presses for production of cocoa butter and cocoa powder in 1828. In the UK in 1847, Joseph Fry was the first to produce a plain eating chocolate bar made possible by introduction of cocoa butter as an ingredient (Beckett, 2000). This preceded the development of milk chocolate by Daniel Peters in 1876 and the conche process by Rodolphe Lindt in 1880. Chocolate confectionery is now ubiquitous with consumption averaging 8.0 kg/person per annum in many European countries (Whitefield, 2005).

Primary chocolate categories are dark, milk and white that differ in content of cocoa solid, milk fat and cocoa butter. The outcome is varying proportions of carbohydrate, fat and protein (Table 1.2). Characteristics of the different chocolate types as laid down in Directive 2000/36/EG of the European Parliament and the Council are shown in Table 1.3.

Table 1.2 Composition of the major nutrients in dark, milk and white chocolate (Chan et al., 1994; Suzuki et al., 2011; Indife et al., 2013)

Product	Carbohydrate (%)	Fat (%)	Protein (%)
Dark chocolate	60.5 - 68.1	28.0 - 33.0	4.7 - 6.6
Milk chocolate	40.3 - 59.1	31.3 - 35.1	7.6 - 8.1
White chocolate	52.0 - 58.3	30.9 - 37.0	7.5 - 8.0

Afoakwa et al. (2008a) describes dark chocolate as coated particles of cocoa and sucrose in phospholipids, suspended in cocoa butter with total solid content ranging from 65% to 75%. It has a very dark colour and most often considered by consumers as bitter. White chocolate differs from milk and dark chocolates through the absence of cocoa solids containing antioxidants, thereby reducing product shelf-life (Beckett, 2000; Whitefield, 2005). Milk chocolate on the other hand contains the entire ingredients (cocoa solids, milk powder, sugar, cocoa butter, flavouring) used to make chocolate. Despite high lipid and sugar contents, chocolate consumption makes a positive contribution to human nutrition through

provision of antioxidants, principally polyphenols including flavonoids such as epicatechin, catechin and notably the procyanidins (Beckett, 2000; Whitefield, 2005). Chocolates also contain minerals, specifically potassium, magnesium, copper and iron (Holland et al., 1991). Differences in sensory characters of chocolate can be attributed to the use of different cocoa types, variations in ingredient proportions, use of milk crumb instead of milk powder, blending techniques and processing methods (Jackson, 1999).

Table 1.3 Characteristics of dark, milk and white chocolate as laid down in Directive 2000/36/EG of the European Parliament and the Council.

Chocolate type	Directive
Dark chocolate	<ul style="list-style-type: none"> Minimally 18% cocoa butter Not less than 35% total dry cocoa solids, minimally 14% dry non-fat cocoa solids
Milk chocolate	<ul style="list-style-type: none"> Not less than 25% total dry cocoa solids, minimally 2.5% dry non-fat cocoa solids Not less than 14% dry milk solids obtained by partly or wholly dehydrating whole milk, semi- or full-skimmed milk, cream, or from partly or wholly dehydrated cream, butter or milk fat, minimally 3.5% milk fat
White chocolate	<ul style="list-style-type: none"> Not less than 20% cocoa butter Not less than 14% dry milk solids obtained by partly or wholly dehydrating whole milk, semi- or full-skimmed milk, cream, or from partly or – wholly dehydrated cream, butter or milk fat, of which not less than 3.5% is milk fat

1.2 World consumption of chocolate

Cocoa is mainly consumed as chocolate confectionery, chocolate coated products (biscuits, ice creams) or in other food products containing cocoa powder including beverages, cakes, and snacks (ICCO, 2012), making chocolate the most commonly craved food. Data published by the Association of the Chocolate, Biscuit & Confectionery Industries of the E.U. (CAOBISCO) in July 2012 show that consumption of all chocolate confectionery products in countries for which statistics are available for the 2002 – 2010 period (which include most of the traditional leading cocoa consuming countries) increased by 10%, an average annual growth rate of 1.2%. Figure 1.1 illustrates the evolution in chocolate consumption in the top

six chocolate consuming countries with the chocolate market still dominated by consumers from Western Europe and North America.

In value, the global chocolate confectionery retail market experienced a significant appreciation, rising from US\$52 billion in 2002 to US\$102 billion in 2011 according to data published by Euromonitor (2012), representing an increase of nearly eight per cent per annum. Increases in standards of living, the development of new products and the use of advertizing and promotional campaigns have contributed to the rise in chocolate confectionery consumption in most regions and countries (ICCO, 2012).

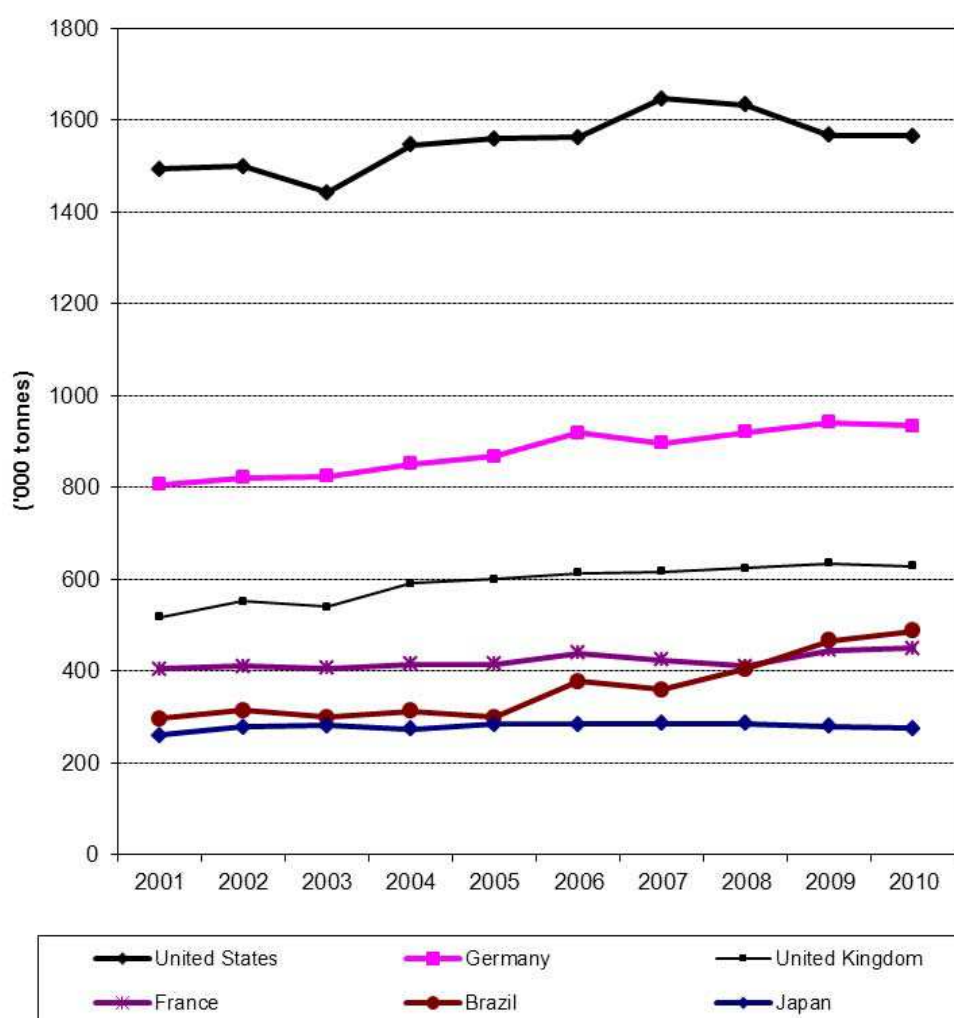


Figure 1.1 Chocolate consumption trend in the top six chocolate consuming countries (ICCO, 2012)

Although overall chocolate consumption in the mature markets increased at a relatively slow pace in volume, the cocoa and chocolate market has witnessed in recent years some changes in consumers' perception of the benefits of chocolate products on human health leading to an increase in the consumption of "premium" chocolate. On the high end of the mature market, chocolate aficionados are asking for single estate and origin "high cocoa content" products with their own distinctive flavours. In particular, consumers seem to have embraced the idea of dark high cocoa content chocolate as an affordable luxury. Chocolate manufacturers have noticed the changing tastes and even companies traditionally known for milk chocolate products have been introducing new dark and high cocoa content products. Innovation is also being used to appeal to consumers in the saturated markets: new flavours, new packaging and new sizes for health-conscious consumers (ICCO, 2012). On the other side of the market, the youthful populations of the BRIC countries (Brazil, the Russian Federation, India and China) with their disposable incomes are a major driving force behind the growth in chocolate consumption. Manufacturers are catering for specific consumer tastes: Cadbury India reported it's highest-ever sales and net profit in 2011, after ramping up distribution and adding new products from its portfolio. In China where the chocolate confectionery market is very young and products are often bought as gifts, the market is currently experiencing very rapid growth, mainly thanks to the growing middle class of more than 300 million people. With the Indonesian market also expanding at a very rapid pace, the Asian market is expected to hold a 20% share of the global market by 2016 according to Mintel (ICCO, 2012).

Figures for consumption of chocolate products in 2010/2011 review period revealed Germany had the highest per capita consumption with 11.56 kilograms per head followed by Switzerland (10.51 kilograms per head) with Bulgaria consuming only 1.26 kilograms per head. Others include United Kingdom (9.72 kg/head), Norway (9.44 kg/head), USA (5.29 kg/head), Australia (4.51 kg/head), Brazil (2.93 kg/head) and Japan (2.09 kg/head). The per capita consumption of chocolate confectionery by country is shown in figure 1.2.

Recently, emphasis has been placed on nutritional benefits arising from chocolate consumption. In the European and American diet, cocoa solids represent a significant source of polyphenols which are discussed for being beneficial in heart and vascular protection through their antioxidative activity (Arifdjohan & Savaiano, 2005; Ding et al., 2006; Engler &

Engler, 2006). Alberts and Cidell (2006) also pointed on specific consumer's attitudes in central Europe where chocolate is regarded as an important food, which undoubtedly affect marketing strategies. New product development and 'functional foods' with wholesome ingredients are thus playing an important role in the upward trend of the confectionery market. Subsequently, the demand for dark and high cocoa content chocolate in particular has surged in response to these positive findings (ICCO, 2012).

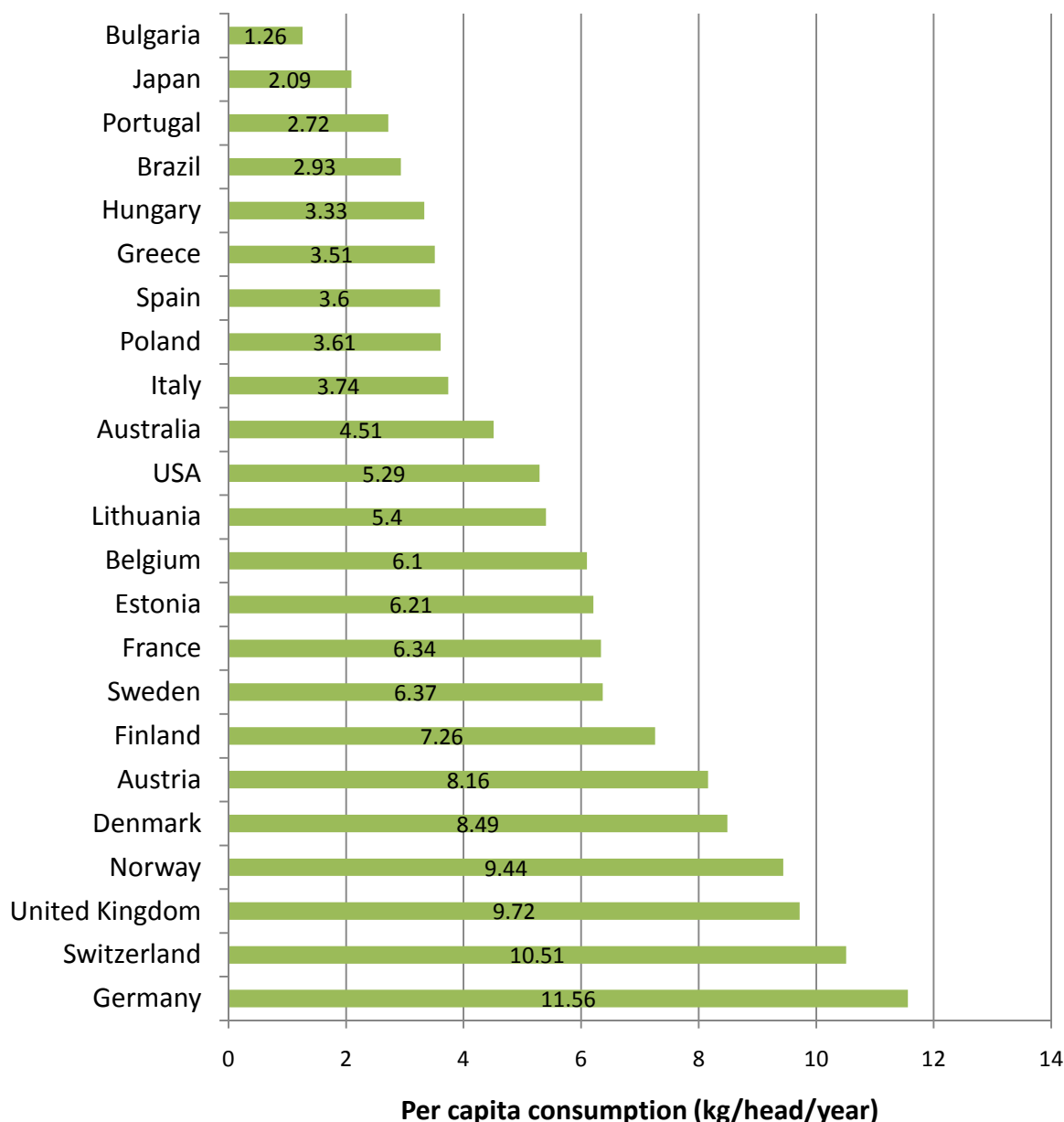


Figure 1.2 Per capita chocolate confectionery consumption (including white chocolate) by country for 2010/2011 review period (International Confectionery Association [ICA], 2012)

1.3 Chocolate manufacturing processes

Chocolate manufacturing processes generally share common features (Figure 1.3) such as mixing, refining and conching of chocolate paste. The outcome sought is smooth textures of products considered desirable in modern confectionery and elimination of oral perceptions of grittiness. Chocolate manufacturing processes (Beckett, 2000; Awua, 2002; Whitefield, 2005) differ due to variations in national consumer preferences and company practices. Specifications depend on type of chocolate and its intended use (Jackson, 1999).

Chocolates contain cocoa liquor, sugar, cocoa butter, milk fat and milk powder (depending on product category). A mix of sugar, milk solids and cocoa liquor is refined to particle size $<30\text{ }\mu\text{m}$ normally using a combination of two and five-roll refiners (Beckett, 2000; Afoakwa, 2010). Only a part of the cocoa butter (20% - 25%) is added at this stage enough to produce a rough paste that can be easily refined in the next process (Dand, 1999). Final particle size critically influences the rheological and sensory properties. A refiner consists of a vertical array of hollow cylinders temperature controlled by internal water flow, held together by hydraulic pressure. A thin film of chocolate is attracted to increasingly faster rollers until removed by a knife blade. Roller shearing fragments solid particles and coats new surfaces with lipid so that the surfaces become active, absorbing volatile flavour compounds from cocoa components (Afoakwa, 2010). Refiners, in summary, do not only effect particle size reduction and agglomerate breakdown but distribute particles through the continuous phase coating each with lipid (Afoakwa, 2010).

Refined mixtures then move into conching, a process that contributes to development of viscosity and final texture and flavour. This is the endpoint for manufacture of bulk chocolate, whether dark or milk. Conching is normally carried out by agitating chocolate at $>50\text{ }^{\circ}\text{C}$ for some hours (Beckett, 2000). Generally a two-stage process is used. The first stage converts the flaky product obtained after refining into a paste by mechanical or heat energy, driving off moisture and undesirable volatiles, effects oxidations and distributes lipids through a continuous fat phase. The second stage converts the thick paste into a free flowing liquid through addition of cocoa butter and lecithin. Conching conditions show interactions between time and temperature so that higher temperatures reduce processing time.

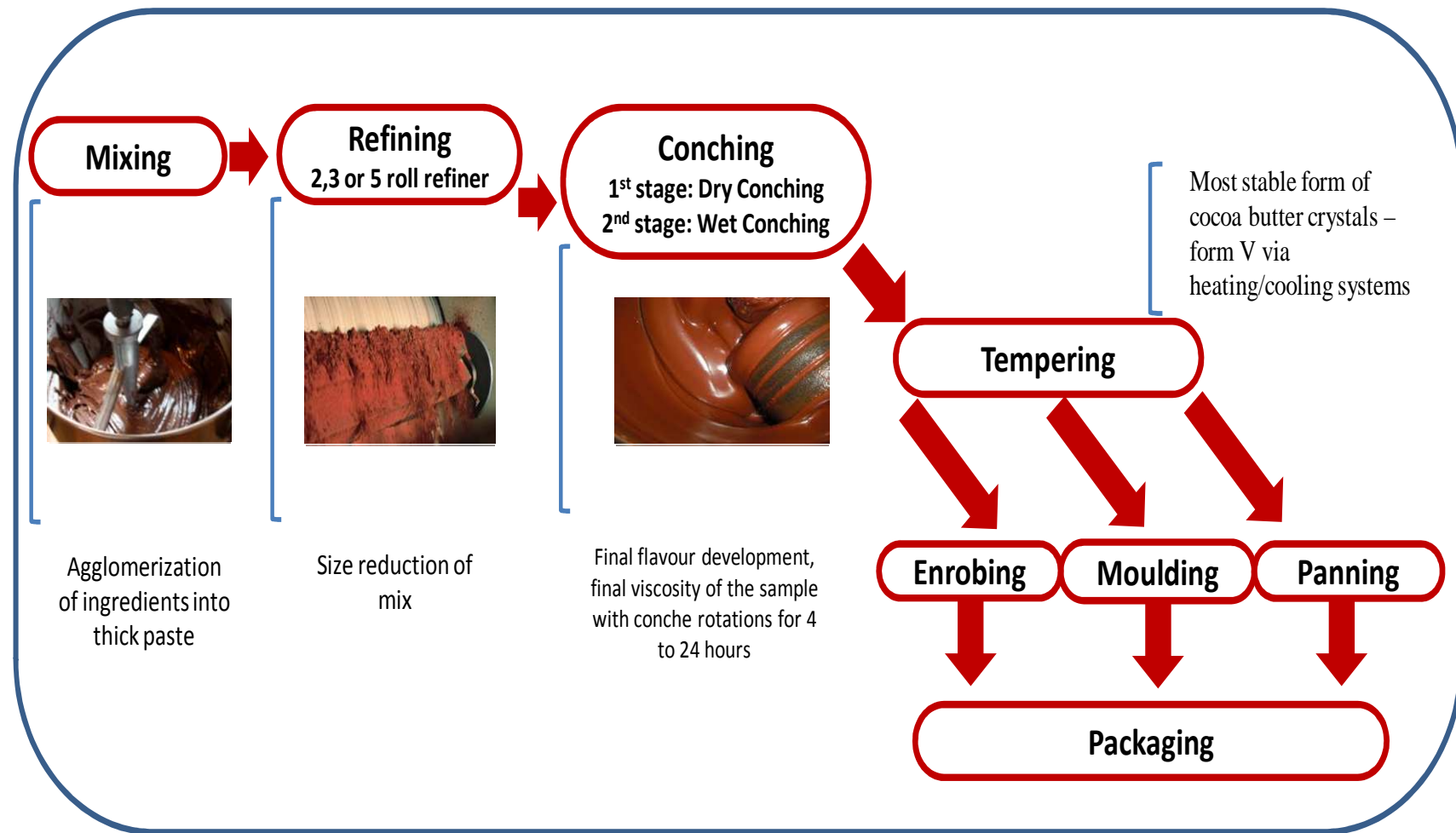


Figure 1.3 Processing steps for chocolate manufacture

Conching conditions for crumb milk chocolate are 10-16 h at 49-52 °C but 16-24 h at 60 °C for milk powder chocolates; temperatures above 70 °C lead to changes in cooked flavours (Beckett, 2000; Awua, 2002; Beckett, 2003; Whitefield, 2005). Dark chocolates are typically conched at higher temperatures (70 °C - 82 °C) (Minifie, 1989; Awua, 2002). Conditions may be modified (generally shortened) by pre-treatment of chocolate liquors as thin films at temperatures >100 °C (Minifie, 1989; Afoakwa et al., 2007).

Prawira and Barringer (2009) investigated the influence of increasing conching time on particle size and sensorial properties of chocolate. They concluded that by increasing the conching time up to 76h, the particle size decreases and the chocolate has a smoother mouthfeel. Bolenz et al. (2003) put forward that with milk chocolate, flavour development is less important than developing the desired rheological and mouthfeel properties. Therefore they suggested the use of shorter conching times when starting from high quality raw materials that contain as little water as possible. The molten product obtained at the end of this stage requires tempering, a technological process for controlling crystallization needed in the generation of the desired β_v crystal form of cocoa butter in the finished product.

1.4 Fat (*cocoa butter*) crystallization during chocolate manufacture

Cocoa butter is a natural fat obtained from cocoa seeds (*Theobroma cacao*). It is commonly used as an essential ingredient of chocolate and other confectionery products due to its specific physical and chemical properties. Cocoa butter is composed mainly of triacylglycerols (TAG) and dominated by three forms which account for 79–89% of the TAG composition: 1-palmitoyl-2-oleoyl-3-stearoyl glycerol (POSt), 1,3- distearoyl-2-oleoyl glycerol (StOSt) and 1,3 dipalmitoyl-2- oleoyl glycerol (POP), where P, O and St stand for palmitic, oleic and stearic acid, respectively (Ray et al., 2012).

The simple triglyceride composition confers the short melting behaviour which is appreciated by consumers. Cocoa butter is a solid at room temperature (below 25 °C) and at body temperature (~ 37 °C) it is liquid (Mohd Omar et al., 2013). Cocoa butter crystallizes in a number of polymorphic forms, each having a different melting point and crystal structure (Wille & Lutton, 1996). A mixed nomenclature frequently used in literature based on 6

roman numerals (I -VI) for 6 polyforms (Willie and Lutton, 1996) and 5 greek letters has been reported by Ray et al. (2012). The melting temperature for form I or γ , II or α , III and IV or β' , V or β_v and VI or β_{vi} is 17.3 (I), 23.3 (II), 25.5(III) and 27.5(IV), 33.8 (V) and 36.3 °C (VI), respectively. The notation by Greek letters does not distinguish between forms III and IV (Ray et al. (2012). Polymorphic triglyceride forms differ in distance between fatty acid chains, angle of tilt relative to plane of chain end methyl group and manner in which triglycerides pack in crystallisation (Talbot, 1999).

Form β_v is the desired form for chocolate manufacture as the melting temperature of this form is high enough to maintain the chocolates in solid form at room temperature and low enough for the chocolate to melt in the mouth (Stapley et al., 1999). It also provides the desired snap, gloss and shelf life (Beckett, 2008). For chocolate to be in an appropriate polymorphic form, tempering is crucial, influencing final quality characteristics such as colour, hardness, melting and shelf-life characteristics. The most frequently applied procedure for obtaining stable form β_v involves subjecting the chocolate to a well-defined temperature programme under the action of shear which induces the formation of a small proportion (1–3 vol%) of seed crystals. Through this process the remaining fat solidifies around the seeds which induces the correct polymorphic form (Seguine, 1991; Stapley et al., 1999; Talbot, 1999). This “conventional” tempering has four key steps: (i) complete melting at 50 °C (ii) cooling to the point of crystallisation at 32 °C (iii) crystallisation at 27 °C; and (iv) melting out unstable polymorphs at 29–31 °C (Talbot, 1999) (Figure 1.4).

Tempering sequence is a function of recipe, equipment and the final purpose. Before the use of tempering machines chocolate used to be hand-tempered and this method is still occasionally used by chocolatiers, who produce relatively small quantities of hand-made confections. Current tempering machines consist of multistage heat exchangers through which chocolate passes at widely differing rates making it difficult to identify optimum conditions. Time-temperature combinations are of paramount importance in process design and in continuous tempering, molten chocolate is usually held at 45 °C then gently cooled to initiate crystal growth. Untempered chocolate is soft and not effectively demoulded. Form β_{vi} is difficult to generate although formed on lengthy storage of tempered chocolate accompanied by fat bloom.

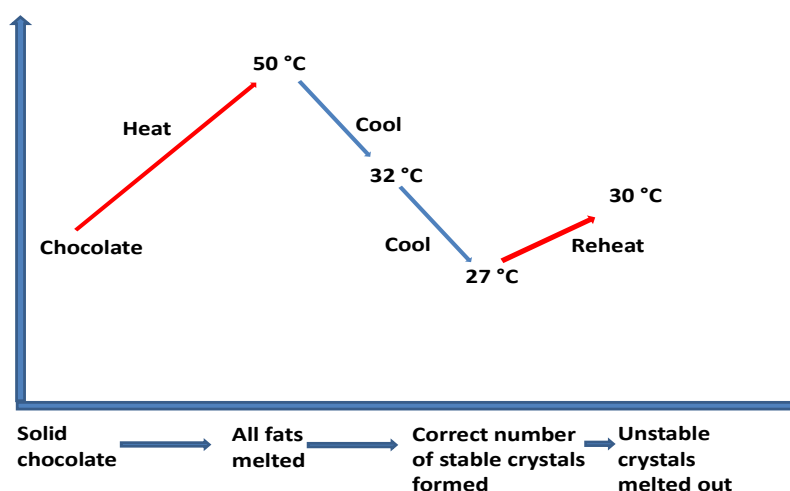


Figure 1.4 Tempering sequence during lipid crystallisation in chocolates

This can be seen as undesirable white or streaky grey-white spots on the chocolate surface. Fat bloom formation on chocolate products can be related to mainly three factors, i.e., poor tempering (polymorph and crystallization control) during production, storage at high temperatures ($>23\text{ }^{\circ}\text{C}$) and/or fluctuating temperature leading to melting and re-crystallization of cocoa butter, and migration of filling oil into the surrounding chocolate shell in the case of center-filled chocolate products, which promotes crystal growth (Dahlenborg et al., 2012). Zeng (2000) presented a novel pre-crystallisation technique for producing well-tempered chocolate by homogeneously mixing 0.2–2% (w/w) of cocoa butter crystals in the most stable form β_{VI} with pre-cooled chocolate. This process results in a large number of nuclei being present which provides the basis from which the fat crystals grow. Although the seed crystals are in polymorphic form β_{VI} , the surrounding chocolate solidifies in the preferred form β_V (Zeng et al., 2002).

The tempering regime for milk chocolate slightly differs from that for dark chocolate due to the influence of milk fat molecules on crystal lattice formation (Haylock & Dodds, 1999). Milk chocolate contains a proportion of butter fat that causes an eutectic effect which prevents bloom formation, results in a lower melting point, softening of texture and lowering of temperature to obtain crystal seed for the tempering process (around $29.4\text{ }^{\circ}\text{C}$ compared to

34.5 °C for plain chocolate) (Haylock & Dodds, 1999). The increasing demand on cocoa butter supply has led to increased costs and an ever rising demand for alternatives to natural cocoa butter. Food industries are therefore keen to find alternative fats to cocoa butter from natural matrices that are denoted as cocoa butter replacers (CBRs), cocoa butter equivalents (CBEs) and cocoa butter substitutes (CBSs). These cocoa butter alternatives may also find application in the chocolate industry.

1.5 Quality parameters in chocolate manufacture

Flavour, appearance and texture are three very important quality characteristics of chocolates. These characteristics are defined by measurable variables like rheology, particle size distribution, hardness, fat crystallization behaviour, gloss and colour, which can be determined by means of instrumental techniques. Sensory analysis can also be performed to define human perception.

1.5.1 Flow properties

Flow properties of chocolate are important in manufacturing process for obtaining high-quality products with well-defined texture (Servais et al., 2004). Molten chocolate represents a suspension of sugar and cocoa solids and, eventually, milk powder particles (depending on type), all reduced in size by roll refining to < 30 µm, in a liquid matrix of mainly cocoa butter (Shantz & Rohm, 2005). Rheologically, molten chocolate exhibits a non-Newtonian behaviour which is conventionally defined by a yield stress and a plastic viscosity. The yield stress is related to the amount of energy needed to start flow and the plastic viscosity to the energy required to maintain flow. The flow properties of molten chocolate are affected by processing (refining, conching and tempering) as well as composition (amount of fat, amount and type of emulsifiers, particle size distribution) (Vavreck, 2004; Schantz & Rohm, 2005; Afoakwa et al., 2009b). These properties not only determine the efficiency of stages such as mixing and pumping but also play an important role in the different chocolate applications such as enrobing, shell formation and molding steps (Servais et al., 2004). As such, controlling the rheological properties of chocolate is paramount.

Of techniques for characterising rheological properties, the International Confectionery Association (ICA, previously IOCCC) suggests the use of rotational viscometers with concentric cylinders (bob and cup geometry) and the Casson equation (International Confectionery Association, 2000; Bouzas et al., 1995; Sokmen & Gunes, 2006; IOCCC, 1973) with measurement of stress and viscosity at shear rates between 2 s^{-1} and 50 s^{-1} using up and down curves preceded by a pre-shear at 5 s^{-1} of $>5 \text{ min}$ (Servais et al., 2004). Important are the rheological models of Herschel-Bulkley, Casson and Bingham (Chevalley, 1999; Beckett, 2000; Sokmen & Gunes, 2006), following the equations:

Herschel-Bulkley: $\tau = \tau_0 + \eta_{pl} \cdot (\dot{\gamma})^n$ (Eqn. 1.1)

Casson: $\sqrt{\tau} = \sqrt{\tau_{CA}} + \sqrt{\eta_{CA}} \cdot \sqrt{\dot{\gamma}}$ (Eqn. 1.2)

Bingham: $\tau = \tau_0 + \eta_{pl} \cdot \dot{\gamma}$ (Eqn. 1.3)

(τ : shear stress (Pa); τ_0 : yield stress (Pa); η_{pl} : plastic viscosity (Pa.s); τ_{CA} : Casson yield value (Pa); η_{CA} : Casson plastic viscosity (Pa.s); $\dot{\gamma}$: shear rate (s^{-1}); η : viscosity of the suspension (Pa.s); n : flow viscosity index).

Since 1973, the ICA has accepted rheological measurement of molten chocolate using rotational viscometers with concentric cylinders (bob and cup geometry) and Casson equation of the parameters (IOCCC, 1973; Bouzas & Brown, 1995). The basis for change in 2000 were results from an inter-laboratory study (Aeschlimann & Beckett, 2000), that concluded that the Casson's mathematical model employing only a small set of parameters was limited in accuracy as, at lower shear rates, rheology data do not fit the Casson equation well. The outcome was a low degree of repeatability inter-laboratory analyses, and ICA thus recommended use of interpolation data for chocolate viscosity. Servais et al. (2004) noted this strategy was simple, accurate and readily applicable to different systems, given a basis of relevant information. In the USA, the current National Confectioners Association/Chocolate Manufacturers Association (NCA/CMA) method to characterize chocolate rheological properties is to extrapolate concentric cylinder flow data using the Casson equation (Baker et al., 2006).

The role of surfactants on chocolate flow properties

Chocolate has a continuous fat phase in which sugar, being hydrophilic and lipophobic, will not dissolve so surfaces have to be coated with fat. This does not occur readily and a surface active agent is beneficial and allows the fat content of the chocolate to be reduced while maintaining desirable flow properties. Lecithin, a by-product of soya-oil production is the most widely applied emulsifier in chocolate manufacture. It is a mixture of natural phosphoglycerides (Minifie, 1980). In chocolate, the most surface active component of crude lecithin (mainly oleic C18:1 and palmitic acid C16:0) is believed to be phosphatidylcholine (PC) (Vernier, 1997). According to Chevalley (1999), the addition of 1–3 g/kg soy lecithin causes the same viscosity-reducing effect as approximately 10 times this amount of added cocoa butter, thus allowing to reduce production costs by saving cocoa butter. The viscosity reducing effect of lecithin is mainly due to its action on sugar: the lipophobic head of lecithin binds on the sugar particles, while the lipophilic tail group aids the flow by rendering the sugar more hydrophobic (Harris, 1968). At more than 0.5% lecithin, an increase in yield value occurs (Chevalley, 1999; Rector, 2000; Schantz & Rohm, 2005) which is linked to the formation of bilayers around the solid particles or to the formation of lecithin micelles, both reducing the effectiveness of the emulsifier (Beckett, 2009). In the EU, no maximum level is specified (*quantum satis*) for the use of lecithin in cocoa and chocolate products (EU Directive 95/2/EC).

Other surface active agents such as ammonium salts of phosphatide acids (YN) and polyglycerol polyricinoleate (PGPR) have been developed for use in chocolate manufacture (van Nieuwenhuyzen & Szuhaj, 1998). YN is a synthetic lecithin prepared from rape seed oil and is therefore more constant in composition than the natural soy lecithin. Unlike lecithin, no increase of yield value occurs with increasing YN content above 0.5%. PGPR is prepared by partial esterification of condensed castor oil fatty acids with polyglycerol (Wilson et al., 1998). Legally approved within the EU, PGPR can be used in cocoa-based confectionery at up to 0.5% (Rector, 2000). It does not have large effects on plastic viscosity but can reduce yield value by 50% at 0.2% or remove it at about 0.8% (Rector, 2000; Schantz & Rohm, 2005). Rector (2000) reported that chocolate with 35% cocoa butter content has a similar yield value to that containing 32% cocoa butter and 0.1 % PGPR. PGPR coats solid particles and

with higher molecular weight, extends further into the lipid continuous phase, producing a better steric stabilization (Vernier, 1998). In contrast to lecithin, PGPR in chocolate does not structure within the suspension but increases the continuous phase volume fraction and binds residual water in chocolate making it unavailable to hydrate and swell the solid particles (Rector, 2000; Schantz & Rohm, 2005). The advantages of PGPR may be increased by combining it with lecithin. Depending on the application, the optimum lecithin:PGPR blends are given with 2:1 (Schuster, 1985), 2.5:1 (Hasenhuettl & Hartel, 1997) and 3:1 (Hofs & Taschke, 2002). Adding PGPR to chocolate containing 0.5% of lecithin gives a further decrease in yield value and only slight increase in plastic viscosity (Rector, 2000). Schantz and Rohm (2005) concluded that irrespective of the type of chocolate, yield stress minima can be obtained by using emulsifier blends with a lecithin to PGPR ratio of 30:70.

1.5.2 Particle size distribution (PSD)

Particle size distribution is a key determinant of the flow (rheological) properties of chocolates with a direct influence on sensory character. Larger particle sizes are important for mouth-feel notably grittiness but smaller particles influence flow properties (Beckett, 2000). Traditionally, continental European chocolate has been described as having a fineness of 15 -22 μm particle diameter and in North America 20-30 μm (Jackson, 1999). However, with increased globalisation of the industry, traditional differences have begun to blur with specifications becoming much more product specific.

Most chocolate products have bimodal and trimodal particle size distribution. Figure 1.5 shows a typical particle size distribution of commercial enrobing mass. Chevalley (1994) showed that as the particle size distribution becomes broader in the direction of smaller particles, increase in casson yield stress is observed. This is due to an increase in the internal surface area, and therefore either the number of bonds or frictional contact between the particles is higher. Afoakwa et al. (2008) also reported consistent decrease in apparent viscosity of dark chocolate with increase in particle sizes, a trend noted at all fat contents. The authors explained that as particle size increases, the packing ability of solids is restricted leading to fewer particle-particle interactions hence the decrease in viscosity (Afoakwa et al., 2008). Servais et al. (2002) showed that varying the blend ratio of chocolates of fine ($d_{4,3} = 8.5 \mu\text{m}$) and coarse ($d_{4,3} = 17.0 \mu\text{m}$) particles influenced relationship between packing

fraction and plastic viscosity, with ratio of 60% coarse particles to 40% fine particles recording the lowest viscosity.

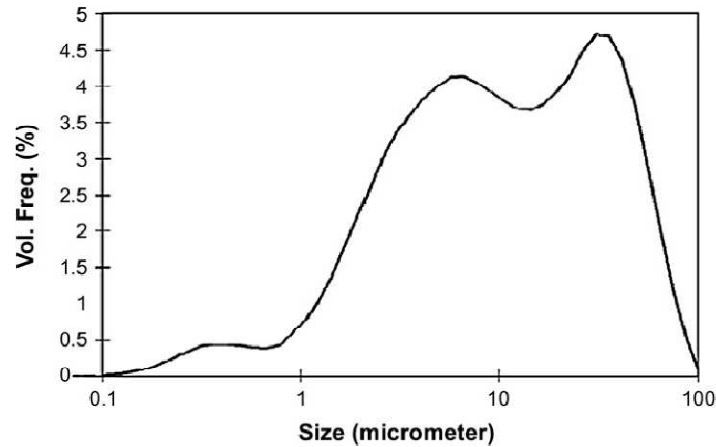


Fig. 1.5. Particle size distribution of commercial enrobing mass during chocolate manufacture.

Particle size distribution has been used as a tool to control consistency of solid-liquid mixtures to aid pumping and mixing of molten milk chocolate (Mongia & Ziegler, 2000), transportation, atomisation and grinding of foods of high solid content in milk suspensions (Seaseaw et al., 2005). Optimisation of particle size distribution in chocolate requires consideration of palate sensitivity. For example, there is a maximum particle size of 30 μm , else a product is perceived as 'gritty or coarse' in the mouth and chocolate milled to a maximum particle size of 20 μm will have a creamier taste and texture than that with 30 μm (Beckett, 2000). Despite the application of particle size distribution in determining suspension flow properties, Awua (2002) and Whitefield (2005) explained that it is not the only factor influencing rheological characteristics. Thus, the general principles of modification of suspension viscosity by changing the particle size require review of a system's properties and compositional factors that contribute to the changes in physical properties, flow behaviour and sensory character of chocolate.

1.5.3 Texture and Appearance

Chocolate texture and appearance are key attributes in consumer choice and acceptability even though flavour is frequently judged important in product identification (Beckett, 2003; Whitefield, 2005). Texture is a combination of the physical structure of a material and its mechanical and surface properties, also known as mouthfeel (Dobraszczyk and Vincent, 1999). Primary characteristics include hardness, cohesiveness, viscosity, springiness and adhesiveness, while secondary parameters consist of fracturability, chewiness and gumminess. Although texture perception is a dynamic oral process before and during mastication, individuals also perceive texture through vision, touch, and hearing (Heath & Prinz, 1999; Kilcast, 1999; Wilkinson et al., 2000). Chocolate has a unique mouthfeel because cocoa butter has a narrow melting point very close to body temperature. As bloom forms (both fat and sugar), the texture is changed and a difference is perceived (Andrae-Nightingale et al., 2009). Chocolate texture can also be evaluated by instrumental measurements often rationalized as cheap, efficient and objective replacements for sensory evaluations (Lawless & Heymann, 1998) with statistically significant correlations (Mohamed et al., 1982; Christensen, 1984; Meullenet et al., 1997; Rosenthal, 1999; Bourne, 2002). The most common method of instrumental texture analysis is a texture probe or texture analyzer (Andrae-Nightingale et al., 2009).

Visual information characterizing objects, including gloss, colour, shape, roughness, surface texture, shininess and translucency, is summarized into appearance attributes. Briones et al. (2006) concluded that these emerge from complex interactions of incident light, optical characteristics and human perception. Relevant information can be acquired from modern technologies such as computer vision and calibrated colour imaging analysis, HunterLAB and CIELAB models (Lawless & Heymann, 1998; Jahns et al., 2001; Hatcher et al., 2004; Briones & Aguilera, 2005). Such LAB-based models provide close descriptions of colour attributes (Lawless & Haymann, 1998; Taylor & Hort, 2004) although Thai and Shewfelt (1991) found that L (lightness), C (chroma) and H (hue angle) from HunterLAB data were better correlated. Given that chocolates should meet prior acquired consumer expectations, appearance attributes can have significant commercial implications.

1.5.4 Microstructure

The processability, texture, flavour and keeping qualities of food is controlled not just by chemical composition but also by how the various ingredients are distributed and interact at the nano – and microscopic length scales (Aguilera, 2000). Microstructure is a fundamental variable influencing transport phenomena and physical properties of foods, determining perceived quality in terms of mechanical and sensorial attributes (Kulozik et al., 2003). From a colloidal perspective, chocolate consists of micron- scale particulates (sugar crystals, cocoa particles, and milk powder) dispersed within a solid/liquid fat phase consisting primarily of cocoa butter (CB). The microstructure of molten dark chocolate is illustrated in Figure 1.6. The interactions between the suspended particles and the continuous phase provide information about the existing network and consequently can be associated with the properties and characteristics of the dispersions (Sato & Cunha, 2009).

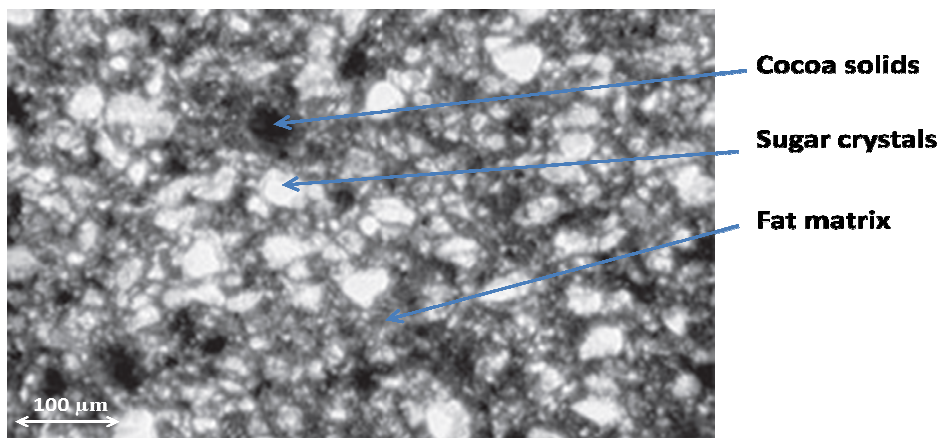


Figure 1.6. Light microscope image of dark chocolate showing the distribution of solid particles in the fat continuous phase.

Various microscopy techniques have been applied to study chocolate microstructure. Afoakwa et al. (2008) applied scanning electron microscopy (SEM) to study the effects of tempering and fat crystallization behaviours on chocolate microstructure. Micrographs of optimally- and over-tempered chocolates showed spatial distribution of dense crystalline network with well defined interparticle connections suggesting stable β -polymorph (Fig. 1.7A-B) whereas under-tempering resulted in formation of solid bridges with weak and fewer inter-crystal connections within the chocolate structure (Fig. 1.7C).

Glicerina et al. (2014) utilized the Environmental scanning electron microscopy (ESEM) and

reported wide variations in the network structure of dark chocolate at each processing step. Chocolate manufacturing steps not only affect particle size reduction but also break agglomerates and distribute lipid and lecithin-coated particles through the continuous phase, modifying the microstructure of final chocolate (Afoakwa et al., 2008). Dahlenborg et al. (2012) employed both Polarized light microscopy (PLM) and Confocal laser scanning microscopy (CLSM) to investigate the porous structure of white chocolate, where specific surface imperfections on chocolate were found to be part of a network of pore structures at and beneath the chocolate surface which could be related to oil migration and thus to fat bloom formation. The same technique was used by Bowser (2006) to investigate the effects of emulsifiers and solid particles on cocoa butter crystallisation in tempered and non-tempered chocolate samples.

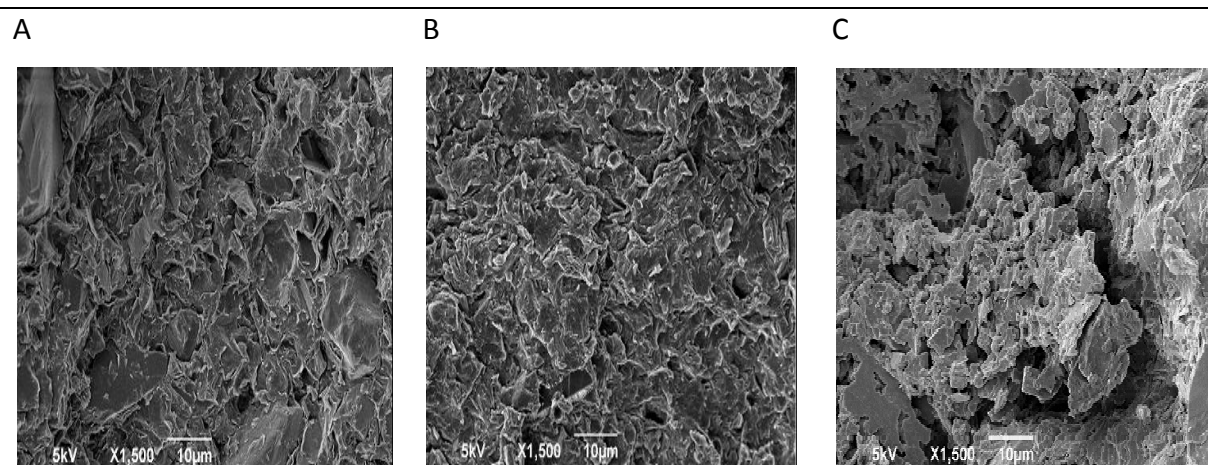


Figure 1.7 Scanning electron micrographs of (A) optimally-tempered, (B) over-tempered and (C) under-tempered (bloomed) dark chocolates (Afoakwa et al., 2008)

Characterising the nature of crystals in chocolate is therefore an important step in quantifying the physical and sensory properties, as the resulting fat crystal network along with the phase behaviour and structural arrangements impact on mechanical, rheological, melting properties and shelf life. The qualitative structural information illustrated by micrographs thus provides a mechanistic explanation for quantitative differences in chocolate properties.

1.5.5 Moisture

Molten chocolate typically has moisture contents of 0.5-1.5%, mainly in the cocoa solids,

that does not affect chocolate flow. Unfortunately, water within chocolate can affect quality, resulting in blooming and reduced snapability (Beckett, 2000). The addition of 3% or 4% of water (by weight) can result in a paste-like chocolate. Greater moisture aggregates sugar particles to form gritty lumps and moisture at sugar particle surfaces increases friction between the particles, resulting in the increase in apparent viscosity. Beckett (2000) stated that for every 0.3% of extra moisture left within the chocolate at the end of conching, the manufacturer must add an extra 1% fat, and because fat is by far the most expensive major component in chocolate, it is important that as much 'free' water is removed as possible. Water at 3-4% increases viscosity and yield value of chocolate markedly (Chevalley, 1999) and viscosity increases up to 20% moisture, after which an aqueous phase is formed (Beckett, 2000). At this stage, a combined emulsion-suspension has formed, the yield value disappears and the viscosity comes to a lower value (Kleinert, 1976).

**Chapter 2: Industrial manufacture of sugar-free chocolates -
Applicability of alternative sweeteners and carbohydrate polymers
as raw materials in product development - a review**

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(Aidoo et al., 2013)

2.1 Introduction

Chocolate is one of the fastest growing products within the confectionery industry and regarded as an affordable luxury even in the face of the economic hardship of recent years. The global chocolate confectionery retail market experienced a significant appreciation, rising from US\$52 billion in 2002 to US\$102 billion in 2011 according to data published by Euromonitor, representing an increase of nearly eight per cent per annum (ICCO, 2012; Palazzo et al., 2011). According to Afoakwa (2010), chocolate's unique texture, flavor and eating pleasure are the main reasons for its expanding consumption throughout the world.

Increasingly, consumers are becoming concerned about the sugar and calorie content as well as the cariogenicity of confectionery products with 'light' and 'sugar-free' products growing in popularity. A food product can assume a "light" or "sugar-free" claim if it provides less than 40 calories per serving or provides less than 0.5g of sugars per serving, respectively (http://www.myfooddiary.com/Resources/label_claims.asp). Consequently, the growing popularity of these products has led to an increased quest for the use of alternative sweeteners in the dairy, confectionery and beverage industries within the past decade.

While the use of sucrose prevails in traditional chocolate industry, numerous nutritive and non-nutritive sweeteners offer new opportunities for the manufacturer. In addition, edible carbohydrates with lower energy contents have been developed which are suitable for inclusion as bulking agents in chocolate manufacture (Rudolf & Stergios, 1995; Afoakwa et al., 2007a). Nutritive sweeteners are ingredients that substitute for both the physical bulk and sweetness of sugar. Products of this type, sometimes called "sugar replacers" or "bulk sweeteners," include the sugar alcohols (also called "polyols") sorbitol, mannitol, xylitol, isomalt, erythritol, lactitol, and maltitol. Trehalose, tagatose and isomaltulose are bulk sweeteners similar in function to the polyols but are actually sugars rather than sugar alcohols (Salminen & Hallikainen, 2002; Kroger et al., 2006; Beckett, 2009). Non-nutritive sweeteners are substances with intense sweet taste used in small amounts to replace the sweetness of a much larger amount of sugar or sucrose. These include acesulfame-K, aspartame, neotame, saccharin, sucralose, alitame, cyclamate, stevia/steviol glycosides (Kroger et al., 2006) and thaumatin.

Today, sugar-free chocolates on the market are more diverse and offer various levels of quality in terms of appearance, texture, taste and flavour dissimilar to that of their sugar counterparts. This review characterizes the major types of alternative sweeteners and carbohydrate polymers used in the food industry and their suitability and applicability to the manufacture of sugar-free chocolates of acceptable quality.

2.2 Functionality of sucrose in chocolate manufacture

Sucrose is the most commonly used sugar in the food industry and it is a popular ingredient to obtain sweetness in human food preparation (Jamieson, 2008). It is extracted from sugar cane or sugar beet and used as an industrial sweetener in baking, drinks, confectionary, jams, jellies and preserves. Sucrose is a disaccharide composed of the chemically linked monosaccharides glucose and fructose with a molecular weight of 342 g/mol and formula $C_{12}H_{22}O_{11}$ (Beckett, 2009). The glycosidic bond is formed between the reducing ends of the monosaccharide, thus classifying it as a non-reducing sugar. Sucrose has high quality sweetness with a clean sweet taste, quick onset and a minimum persistence. It is mainly valued for its sweetness and serves as an important source of energy, providing 394 kcal/100g of refined sugar. Sucrose is also useful as a bulking agent, texture modifier, mouthfeel modifier, flavour enhancer and preservative (Salminen & Hallikainen, 2002; Afoakwa et al., 2007a).

Chocolate is a dense suspension consisting of sugar particles, cocoa solids, and milk powder (depending on type) dispersed in cocoa butter as a continuous phase (Sokmen & Gunes, 2006; Afoakwa et al., 2007a; Beckett, 2009). Sucrose is utilized between 30-60% in chocolate confectionery (Krüger, 1999) and this confers multiple functional properties on chocolate including sweetness, particle size distribution (PSD) and mouthfeel (texture). Its impact on rheological properties is also important for the end product quality (Jeffery, 1999; Afoakwa et al., 2007b). During processing, the components are mixed, refined and conched to attain desired rheological properties. Finally, tempering, cooling and storage are important for the final product texture and melting characteristics. Guinard & Mazzucchelli (1999) noted that sucrose is added to promote sweetness in chocolate but also affects other flavours. Barringer & Prawira (2009) investigated the effect of the amount of sucrose in milk chocolate on consumer preference. Chocolates with 40% sucrose were significantly higher in

chocolate flavor than those with 30% sucrose despite containing less cocoa liquor. The bitterness attribute was also significantly affected by sucrose levels with panelists rating chocolate with 30% sucrose significantly more bitter than chocolate with 44.3 and 50% sucrose. Similar results were obtained by Guinard & Mazzucchelli (1999) where milk chocolates with less sucrose were also rated by a trained panel more bitter than samples with higher sucrose content.

2.3 Alternative sweetening solutions in chocolate manufacture

Sucrose is the conventional sweetening agent prevailing in the traditional chocolate processing industry. The high sugar content of chocolate have led to the search for low calorie, low glycaemic index, healthier alternatives. While sucrose alternatives do not provide a comparable amount of calories, they are generally poor in mimicking the physical attributes of sucrose, i.e. body, mouthfeel and texture (Clayton & Conn, 2005). Alternative sweeteners are successful if they match closely the taste quality of sucrose. Given that all the sucrose needs to be replaced, sugar-free products depending upon the application are usually the most challenging to develop. The different categories of ingredients that may be used are discussed below.

2.3.1 High potency sweeteners

High potency sweeteners (HPSs) are often called high intensity sweeteners. They deliver a sweetness punch from hundreds to thousands of times that of sucrose and are therefore used at levels of “parts per million” (ppm). Many types exist but a handful is approved for use in Europe and the United States. These include saccharin, sucralose, acesulfame potassium, aspartame and neotame (Jamieson, 2008). The technical characteristics of these sweeteners and their regulatory status are summarized in Table 2.1.

2.3.1.1 *Stevia rebaudioside – A*

The leaves of a South American shrub of the Chrysanthemum family that is commonly called Stevia, contain intensely sweet substances sweeter than sugar. A variety of terms have been used to refer to the sweetening agent extracted from this plant including Stevia, Stevioside

Table 2.1 Technical and regulatory status of some high potency sweeteners (Jamieson, 2008; ^aKroger et al., 2006)

	Aspartame	Acesulfame-K	Stevia Rebaudioside A	Thaumatococin	Saccharin	Sucralose	Neotame
Sweetness Potency (times that of sucrose)	180-200	130-250	200-300	2000-3000	300-500	500-700	8000-13000
Taste/Profile	Slow onset, Lingering sweetness Clean sweetness with little to no aftertaste	Quick onset with no significant lingering sweetness. Can have a bitter aftertaste	Moderate to quick onset with little to no lingering sweetness. Potential for bitter or black licorice aftertaste	Slow onset, Lingering sweetness Licorice aftertaste	Quick onset with no significant lingering sweetness. Potential for metallic, bitter aftertaste	Clean sweetness with slow onset and lingering sweetness	Slow onset, lingering sweetness Clean sweetness with little to no aftertaste
Stability	Limited stability at elevated temperature and low pH	Good stability at elevated temperatures and low pH	Good stability at elevated temperatures and low pH	Good stability at elevated temperatures	Good stability at elevated temperatures and low pH	Good stability at elevated temperatures and low pH	Limited stability at elevated temperature
Blending Options	Good synergy with acesulfame-K and saccharin	Good synergy with aspartame and sucralose	Have been used with erythritol	Enhances sweetness of sugar alcohols	Good synergy with aspartame and sucralose	Good synergy with acesulfame-K and saccharin	Good synergy with acesulfame-K and saccharin

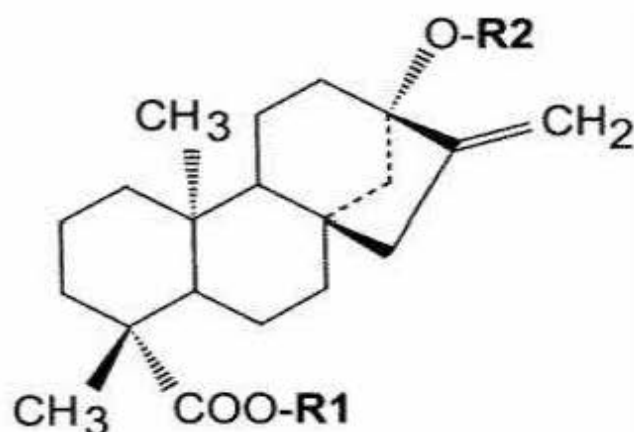
Advantages	Widely used, sweetness profile and cost-effectiveness	Stability and synergies with other HPSs	“natural” status and stability	“natural” status and stability	Cost-effectiveness and stability	Sweetness profile, branding and stability	Sweetness profile and cost effectiveness
Regulatory ^a Status	Food Additive ADI 50mg/kg of body weight/d	Food additive ADI 15mg/kg of body weight/d	Food additive ADI 4mg/kg of body weight/d	Food additive ADI “not specified”	Permitted for use under an interim regulation	Food additive ADI 5mg/kg/d	Food additive ADI 18mg/p/d

^aADI values listed here are those established by the US Food and Drug Administration (expressed in milligrams per kilogram of body weight per day). Neotame is however expressed in terms of milligrams per person per day (mg/p/d).

and Steviol glycosides. The JECFA concluded that the most appropriate name to be used for this extract is steviol glycosides. Most steviol glycoside products sold today consist primarily of stevioside (>80%) or rebaudioside A (>90%). Rebaudioside A is of particular interest because it has the most desirable flavor profile and it is the most stable of the steviol glycosides (DuBois, 2000). Rebaudioside A is considered a natural sweetener approximately 200 to 300 times as sweet as sucrose and has been used for centuries. Steviobioside, rebaudioside B, C, D, E, F and dulcoside A have also been isolated from *S. rebaudiana* Bertoni leafs besides Rebaudioside A and stevioside. Structures of stevioside and related compounds are given in figure 2.1. According to Prakash et al. (2007), it is very difficult to combine a high purity of rebaudioside A with a high recovery because rebaudioside A and its impurities have similar solubilities. Stevia rebaudioside A sweeteners with 60%, 80%, 95% and 97% purity levels are currently available on the European market.

Recently, the launching of new products containing stevia extracts has increased, while those containing artificial sweeteners have decreased as consumers want low-calorie products containing natural sweetening alternatives instead of artificial ones. In December 2008, the United States Food and Drugs Administration (USFDA) accepted the GRAS (Generally Recognized as Safe) status of rebaudioside A and, in 2009, for the mixture of steviol glycosides. In September 2009, the French authorities authorised rebA (>97 % purity) as a food additive, excluding its use as a table top sweetener. However, in November 2011 the European commission finally approved the use of steviol glycosides in a number of food and beverage categories within the European Union which has led to their wide scale use in Europe (Stoyanova et al., 2011). The European Food Safety Authority established an Acceptable Daily Intake (ADI) for steviol glycosides expressed as steviol equivalents of 4 mg/kg bodyweight/day. Conversion factors for steviol glycosides are based on molecular weight of steviol and the glycosides involved. As the molecular weights of the various glycosides are different, JECFA suggested that the concentrations/amounts of steviol glycosides should be expressed as steviol content, which is equivalent to approximately 40% of the stevioside content. For example, the conversion factor for rebaudioside A is calculated by dividing the molecular weight of steviol (318.45 g/mol) by the molecular weight of rebaudioside A (966.43 g/mol). To obtain the steviol equivalents of the different steviol glycosides, their amounts should be multiplied by the conversion factors given in Table 2.2.

Steviol glycosides can be used at maximum levels of 270 mg/l or mg/kg, expressed as steviol equivalents in energy-reduced cocoa and chocolate products (EU Directive 2011/1131/EC). Conditions of use for steviol glycosides in other confectionery including breath refreshing microsweets are presented in Table 2.3. Along with sweetness a bitter taste is also felt in humans.



	Compound name	R1	R2
1	steviol	H	H
2	steviolbioside	H	β -Glc- β -Glc(2→1)
3	stevioside	β -Glc	β -Glc- β -Glc(2→1)
4	rebaudioside A	β -Glc	β -Glc- β -Glc(2→1) β -Glc(3→1)
5	rebaudioside B	H	β -Glc- β -Glc(2→1) β -Glc(3→1)
6	rebaudioside C (dulcoside B)	β -Glc	β -Glc- α -Rha(2→1) β -Glc(3→1)
7	rebaudioside D	β -Glc- β -Glc(2→1)	β -Glc- β -Glc(2→1) β -Glc(3→1)
8	rebaudioside E	β -Glc- β -Glc(2→1)	β -Glc- β -Glc(2→1)
9	rebaudioside F	β -Glc	β -Glc- β -Xyl(2→1) β -Glc(3→1)
10	dulcoside A	β -Glc	β -Glc- α -Rha(2→1)

Figure 2.1 Chemical structures of stevioside and related compounds [Glc, Rha and Xyl represent Glucose, Rhamnose and Xylose, respectively].

Soejarto et al. (1983) believed that the sesquiterpene lactones are responsible for the bitter aftertaste. Phillips (1987) described a European patent which attributes the bitter aftertaste to the presence of essential oils, tannins, and flavonoids.

Table 2.2 Conversion factors for calculating the steviol equivalents of steviol glycosides (EUSTAS, 2007)

To obtain the steviol equivalent of:	Molecular weight (g/mol)	Multiply the amount by:
Stevioside	804.38	0.395
Rebaudioside A	966.43	0.329
Rebaudioside C	950.44	0.334
Dulcoside A	788.38	0.400
Steviolbioside	642.33	0.496
Rebaudioside B	804.38	0.395
Rebaudioside D	1128.48	0.282
Rebaudioside E	966.43	0.329
Rebaudioside F	936.42	0.340

Table 2.3 Authorized usage levels of steviol glycosides in some confectionery products (EU Directive 2011/1131/EC)

E-number	Maximum level (mg/l or mg/kg as appropriate) ^a	Restrictions/exceptions
E 960	270	Only cocoa or dried fruit based, energy reduced or with no added sugar
	330	Only cocoa, milk, dried fruit or fat based sandwich spreads, energy-reduced or with no added sugar
	350	Only confectionery with no added sugar
	2000	Only breath-freshening micro-sweets, with no added sugar
	670	Only strongly flavoured freshening throat pastilles with no added sugar

^a Expressed as steviol equivalents

Nevertheless, stevioside and rebaudioside A are partially responsible for the aftertaste even though the contribution of rebaudioside A is significantly less than that of stevioside (Goyal et al., 2010). This problem can be solved by enzymatic modification of stevioside by pullanase, isomaltase (Lobov et al., 1991), β -galactosidase (Kitahata et al., 1989), or dextrin saccharase (Yamamoto et al., 1994). Rebaudioside A is not only considered the sweetest of

the various compounds found in stevia, it is the one with the least amount of bitterness and off-flavours. However, with increased usage level above that of a 6 percent sucrose-equivalent solution (approximately 400 ppm), bitterness and/or black licorice flavors can possibly be detected. Overall its sweetness profile has been described as having a faster onset than that of other sweeteners such as sucralose, aspartame and neotame, but its duration is much shorter. Regarding stability, rebaudioside A is quite stable in the presence of extremely elevated temperatures, which would be considered a benefit for confections that require such demands. Storage for 24 months in plastic bags resulted in loss of only 1-2% of rebaudioside A (Prakash et al., 2008). The market segment currently utilizing this sweetener seems to be the beverage industry, where being considered “natural” has significant potential (Jamieson, 2008).

2.3.1.2 Thaumatin

Thaumatococcus is an intensely sweet-tasting protein isolated from the arils of *Thaumatococcus daniellii* (Benth), a plant native to tropical West Africa (Ohta, 2011). West African natives have used the fruits of *Thaumatococcus daniellii* (Benth) for centuries to sweeten bread, tea and palm wine. It was only in the last century that samples of the plant and fruit were taken to England and thaumatin, a mixture of sweet proteins extracted from this fruit, was discovered (Gibbs et al., 1996). Thaumatin consists of at least five sweet forms; with two major components (Thaumatococcus I and Thaumatococcus II) and three minor components (Thaumatococcus a, b, and c). All of these forms elicit a sweet taste at approximately 50 nM (Masuda, 2011). Thaumatin is a protein so it has 4 kcal/g. Since it has such high potency relative to sucrose, the amount used is extremely small, and so it does not provide measurable caloric value at usage levels in foods. Thaumatin I and II have similar properties, such as amino acid composition, sweetness, molecular weight (22000 Da) and identical amino acid sequences except for five amino acid residues (Suami et al., 1997). However, Thaumatin I is the most abundant component of the plant. The thaumatin structure utilizes two building motifs: a folded β sheet, or a flattened β "barrel," and the β ribbons and small loops stabilized by disulfide bonds (De Vos et al., 1985). The backbone structure of thaumatin I is shown in Figure 2.2.

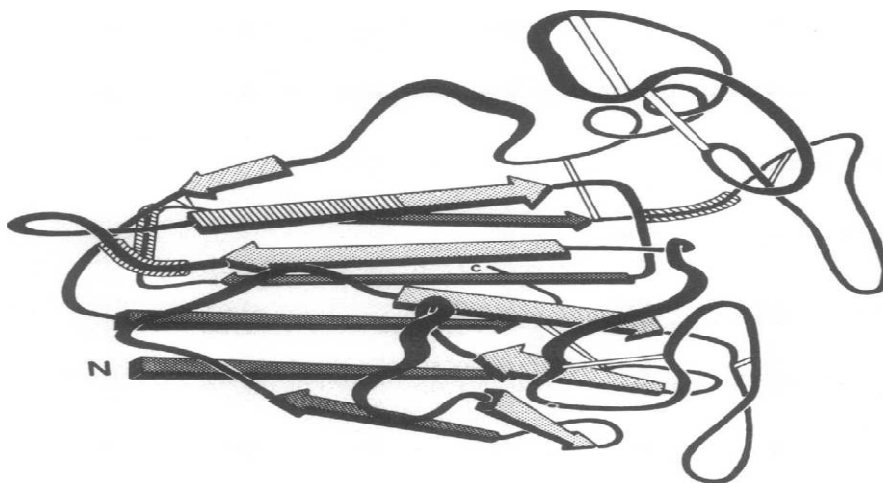


Figure 2.2 Backbone structure of thaumatin I (De Vos et al., 1985)

The main body of the structure consists of two β sheets forming a flattened β barrel. β Strands in the top sheet are shaded light, and those in the bottom sheet are darker. Open bars represent disulfide bonds, and the regions with sequences homologous to monellin are indicated by the hatched marks. The viewing direction is along the crystallographic C axis. At present, thaumatin is the only taste modifying protein (TMP) which is harvested on a large scale and is one of the few intense sweeteners that has undergone extensive safety evaluation (Witty, 1998). The safety of thaumatin has been proven for animal and humans. It does not cause tooth decay and can be used by diabetics. Thaumatin has been found not to exert any toxicity, genotoxicity or teratogenicity (JFECFA, 1985), and the acceptable daily intake (ADI) for man has been evaluated as “not specified” (Hagiwara et al. 2005).

Since 1983, thaumatin has been approved and commercialized as a safe sweetener and flavor enhancer in food (Faus, 2000). Thaumatin has GRAS status in the U.S where it is used as a flavor enhancer, and it is approved as a sweetening agent in other countries, including Australia, Switzerland, and the United Kingdom (Kinghorn et al., 1998). It is approved for use as a sweetener in a small number of food categories in the European Union (Table 2.4). Calvino and Griddo (1998) evaluated the potency of sweetness of aspartame, D- tryptophan and thaumatin using single value and time-intensity measurements. Across the entire sweetness range, thaumatin showed the greatest potency but its long persistence time led to the differentiation of this intense sweetener from the other sweeteners evaluated.

Table 2.4 Authorized usage levels of Thaumatin (E957) as a sweetener in approved food categories in the EU (EU Directive 94/35/EC)

Product type	Maximum use level
Candies without added sugar	50 mg/kg
Candies with cocoa or dried fruits, with reduced energy value or without added sugar	50 mg/kg
Chewing-gum without added sugar	50 mg/kg
Food complements/diet products with vitamins and /or mineral elements, in syrup form or to chew	400 mg/kg
Ice to eat with reduced energy value or without added sugar	50 mg/kg

The onset of sweetness due to thaumatin is relatively slow with a slight liquorice aftertaste. The sensation is not only limited to the front of the tongue but over a large portion of the tongue. The sweetness profile differs from sucrose, thus thaumatin will most probably be used in combination with other sweeteners such as saccharin to mask the bitter aftertaste of the latter (Gibbs et al., 1996). Although this sweet protein mixture is extremely soluble in water, it is not soluble in organic solvents. Even at pH values below 5.5, heat stability above 100 °C has been demonstrated with no loss in sweetness. It is also stable under pasteurization conditions (Gibbs et al., 1996). Thaumatin is currently commercially available as a sweetener, flavor enhancer, additive to pharmaceuticals, chewing gum and animal feeds. It improves the aroma balance and rounds out the flavours in chewing gum. It also enhances the sweetness of sugar alcohols used in foods and can mask agents in medicines and cigarettes, and decrease peppermint threshold by up to 90% (Gibbs et al., 1996).

With the knowledge of the sensorial and technological properties, thaumatin can be explored as a natural sweetener in the production of sugar-free chocolates considering the recent increasing consumer demand for the use of natural sweeteners as alternative sweetening agents for the manufacture of confectionery products. As very little is known about the use and applicability of thaumatin as natural sweetening agent in the development of sugar-free chocolate, it is highly recommended that more advanced studies is conducted on its feasibility of use, alone or in combination with other sweeteners and bulking agents. Replacement of sugar by intense sweeteners poses a serious challenge in

chocolate confections because sucrose fulfils both a structural and sweetening function in these products. Preparation of low sugar or no sugar added products automatically faces the problem of replacing the bulk material in the product which should have at least similar functionality as the replaced sugar (De Baets, 2010). Combination of high potency sweeteners with bulk sweeteners is therefore needed to provide an integral solution for sugar replacement.

2.3.2 Bulk sweeteners

Bulk sweeteners are ingredients that can substitute for both the physical bulk and sweetness of sucrose. Often referred to as “sugar replacers”, bulk sweeteners are constantly being explored industrially for their importance in food applications. Several health promoting effects have been attributed to these ingredients and thus have potential advantages over sugar as food ingredients.

2.3.2.1 Polyols (Sugar alcohols)

Polyols (also known as sugar alcohols) originate from traditional corn syrups modified by reducing the reactive sites (aldehyde or ketone) through catalytic hydrogenation, enzymatic conversion or fermentation. Only the reactive groups are changed so the polyol retains much of the sugar’s structure, bulk and function, making them ideal for 1:1 bulk sugar replacement (Jamieson, 2008). Polyols vary in sweetness from half as sweet to about as sweet as sucrose. They include sorbitol, isomalt, erythritol, maltitol, lactitol, mannitol and xylitol. Their chemical properties are summarized in Table 2.4. The standardized caloric value by European regulators for polyols is 2.4 kcal/g (0 kcal/g for erythritol). Erythritol is thus regarded as not calorific by the EU. The FDA however assigns individual caloric values for polyols as outlined in Table 2.5.

Jamieson (2008) noted that polyols can exhibit a wide range of physical characteristics beyond that of the typical solubility, molecular weight and sweetness. They have other unique properties such as cooling effects which occur when crystalline polyols exhibiting a very negative heat of solution are dissolved in water (often reducing the temperature of their surroundings).

Table 2.5 Key physical, chemical and biological properties of Polyols (sugar alcohols)

Polyol	Caloric value (kcal/g)^a	Properties
Sorbitol	2.6	Derived from glucose; 60% as sweet as sucrose. Good solubility – 70% at 20 °C. Melting point – 97.2 °C. Very hygroscopic and has a cooling effect in crystal form only.
Xylitol	2.4	Derived from xylose. Equal in sweetness to sucrose. It has a solubility of 63% with low melting point of 94 °C. Less laxative and less hygroscopic
Isomalt	2	Derived from sucrose; about 40% as sweet as sucrose. Has solubility of 25% at 20°C which increases with temperature. Melting point between 145 – 150 °C. Not hygroscopic, forms agglomerates with high residual moisture. Less viscous thereby decreasing the viscosity of other polyols.
Mannitol	1.6	Derived from mannose; about 70% as sweet as sucrose. It crystallizes out because of the poor solubility – 18% at 20 °C. Melting point between 165-169 °C. Not hygroscopic but has the highest laxative effect.
Maltitol	2.1	Derived from glucose syrup; 95% as sweet as sucrose. Has a solubility of 62% at 20 °C with a melting point lying between 130-135 °C. Very hygroscopic.
Lactitol	2	Derived from lactose; about 40% sweet as sucrose and exist in 2 forms - monohydrate and anhydrous with melting points of 75 °C and 120 °C respectively. Less hygroscopic than sorbitol or xylitol
Erythritol	0.2	Derived from fermentation of glucose and sucrose by <i>Trichosporonoides megachiliensis</i> ; about 60-80% sweet as sucrose. Has humectant and bulking properties and produces laxative effect upon high consumption.

^aCaloric values as specified by the US Food and Drugs Administration

This may be a welcomed property in applications such as mints or breath-refreshing chewing gum but not necessarily so in chocolates. Most polyols are incompletely digested and poorly absorbed. This is the primary reason why their caloric values are lower than that of sugar. Incomplete absorption may also have disadvantages. Undigested carbohydrate has an osmotic effect, pulling water into the intestine (Kroger et al., 2006). The label statement “excess consumption may have a laxative effect” is therefore required by the USFDA for some products containing sorbitol or mannitol if consumption of the product is likely to result in ingestion of 50 g or more per day of sorbitol or 20 g or more per day of mannitol.

Children may be particularly sensitive to gastrointestinal effects resulting from consumption of relatively small quantities of polyols because of their small body size, (Payne et al., 1997).

2.3.2.2 Tagatose

Tagatose, an isomer of D-galactose and stereoisomer of D-fructose, is a naturally occurring simple sugar that has been established as GRAS (Generally Recognized as Safe) by the FAO/WHO since 2001 for use in food and beverages (Espinosa and Fogelfeld, 2010). It occurs naturally in *Sterculia setigera* gum and small quantities have been found in sterilized and powdered cow's milk, a variety of cheeses, yogurts, and other dairy products (Mendoza et al. 2005). Its molecular formula is $C_6H_{12}O_6$ with a molecular weight of 180 g/mol (Espinosa and Fogelfeld, 2010). The acyclic and β -pyranose structures are shown in Figure 2.3.

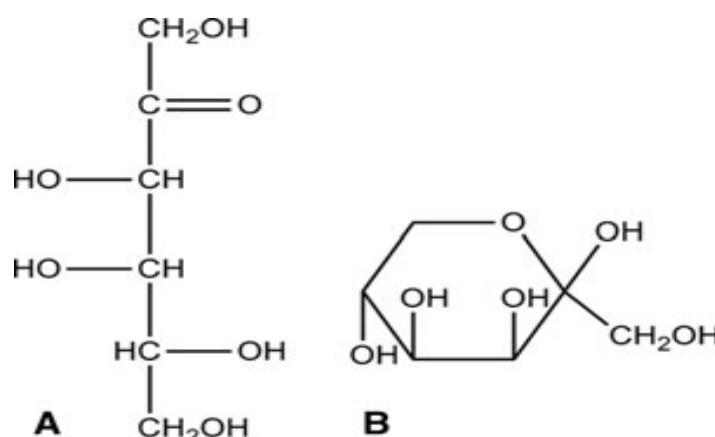


Figure 2.3 (A) acyclic D-tagatose and (B) β -D-tagatopyranose

The FDA approved its use as a food additive in 2003 (FDA, 2003). Interest in tagatose stems from its prebiotic properties (Bertelsen et al., 1999) and as such, its incorporation into foods may provide health benefits to consumers (Venema et al., 2005). Food products containing tagatose are currently on the European market (Damhert, 2009; Luecke and Bell, 2010). Table 2.6 summarizes its health benefits and applications.

Tagatose, like the polyols, has a low caloric value and tooth-friendly properties. According to Bertelsen et al. (2001), only 15 – 20 percent of tagatose is absorbed in the small intestine. The major part of ingested tagatose is fermented in the colon by indigenous microflora, resulting in the production of short-chain fatty acids. A caloric value of 1.5 kcal/g has been

approved by the FDA for labelling purposes. Lee and Storey (1999) compared gastrointestinal tolerance of sucrose, lactitol and tagatose in chocolate. The authors reported that 20 g dose of tagatose given in 40 g of plain chocolate does not result in significant laxation compared to an identical dose of sucrose or lactitol. Consumption of tagatose chocolate did not provoke significantly higher reporting of bloating, colic and flatulence compared to chocolate made with lactitol.

Table 2.5 Health benefits and food applications of tagatose (Oh, 2007)

Health benefits	Applications
Low calorie	Low carbohydrate diets, cereals, health bars, soft drink
No glycemic effect	Diabetic food (type 2)
Anti-halitosis	Anti-hyperglycemic agent, dietary supplement
Prebiotic	Chocolate, candy, chewing gum
Anti-biofilm,	Tooth paste, mouth wash
Flavor enhancement	Yogurt, bakery, milk-based drink, confectionery

Lactitol and tagatose chocolates however did not cause significant symptoms regarded as considerably more than usual (i.e., debilitating) (Lee and Storey, 1999). The sweetening power of tagatose is only slightly less than that of sucrose, and therefore contributes a similar amount of bulk to food products. Tagatose has 92% the sweetness of sucrose when compared in 10% solutions with a sucrose-like taste and no cooling effect or aftertaste. With a sweetness and bulk similar to sucrose, tagatose could be used as a sugar replacer in the formulation of reduced-calorie foods as well as foods low in metabolizable sugars (for example, diabetic foods) (Taylor et al., 2008). The melting temperature of tagatose is 134 °C, and it is stable at pH 2–7. It has high solubility [58% (w/w) at 21 °C] which makes it ideal as a flavor enhancer or fiber in soft drinks and yogurts. It is less hygroscopic than fructose and lower in viscosity [180 cP at 70% (w/w) and 20 °C] than sucrose at the same concentration. As a reducing sugar, tagatose is involved in browning reactions during heat treatment and decomposes more readily than sucrose at high temperatures (Kim, 2004; Levin, 2002).

Despite the U.S. Food and Drug Administration agreeing with the GRAS recommendation of tagatose by an external panel its use is currently limited to specific applications at specified concentrations. Maximum levels of tagatose allowed in specific products were outlined by the FDA. These include 1% in carbonated beverages, 2% in bakery products, 15% in hard candies, and 60% in chewing gum (Rulis, 2001). Tagatose has also been approved by Australia, New Zealand, Korea, Brazil, and South Africa (Skytte, 2006). The mean laxative threshold has been found to be 40 g per meal in human studies, although the intestinal adaptation to its continuous use in humans has not been studied (Levin et al., 1995). The Joint FAO/WHO Expert Committee on Food Additives however found tagatose to be safe and did not specify a maximum acceptable daily intake (WHO, 2005). Although these limitations exist, developing a knowledge base of tagatose functionality in chocolate products would be beneficial to the sugar-free industry.

2.3.2.3 Trehalose

Trehalose, also known as mycose, is a natural alpha-linked disaccharide formed by an α, α -1,1-glucoside linkage of 2 α -glucose units (Figure 2.4). Its molecular formula and weight are $C_{12}H_{22}O_{11}$ and 342.31 g/mol, respectively. Trehalose was first discovered in the early 19th century as a component of the ergot of rye (Wiggers, 1963). It is found naturally in insects, plants, fungi, and bacteria. Commercially grown mushrooms contain 8–17% (w/w) trehalose and substantial quantities are found in honey (0.1–1.9%), mirin (1.3–2.2%), sherries (<10–391 mg/l), brewer's (0.01–5.0%) and baker's yeasts (15–20%) (Aso et al., 1962; Elbein, 1974; Sugimoto, 1995; Van Dijck et al., 1995). Although the α, α isomer is commonly referred to as trehalose, α, β and β, β isomers exist in nature and display physical properties that are quite different from α, α -trehalose (Elbein, 1974). Purified commercial trehalose is usually in the dihydrate form. Trehalose technical qualities, mechanisms of action and natural functions make it possible for its applications in the food, cosmetic and medical industries (Roser, 1991a,b; Colaco and Roser, 1995; Sugimoto, 1995). Trehalose was mainly applied in medicine and cosmetics as its use in the food industry was limited by cost (Sugimoto, 1995). With the advent of new manufacturing processes, the cost of production of trehalose has been dramatically reduced allowing its use in a wide variety of foods. Table 2.7 lists some

current and foreseen applications of trehalose in the food industry. Trehalose popularity according to Portmann and Birch (1995) may be due to its lower sweetness and longer persistence (in sweetness) in comparison with sucrose.

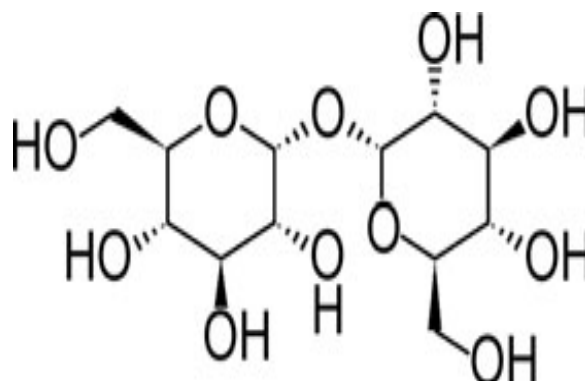


Figure 2.4 Chemical structure of Trehalose

Table 2.7 Food applications of trehalose (Schiraldi et al., 2002)

Type of food	Examples
Dried food	Egg, vegetables, fish, meat, cereal, beans, powder mil, fruits, soup
Frozen food	General cooked food
Confection	Candy, gum and chocolate
Confectionery	Cake, baked confectionery, jam, cream
Beverage	Coffee, tea, fruits juice and diary beverage
Fermented food	Bread and yoghurt
Others	Ice cream, sauce, sweetener and seasoning

Trehalose is almost half as sweet as sucrose and thus can be used in combination with other bulk sweeteners (Portmann and Birch, 1995). A 22.2% solution of trehalose was judged to be about 45% as sweet as sucrose by a Japanese taste panel. In this study, 85% of the panel preferred the taste of trehalose compared to sucrose (Richards, 2002). Portmann and Birch (1995) reported a faster increase in the perceived sweetness of trehalose compared to sucrose (by a factor of 2.5) as the concentration of the solutions increased from 2.3 to 9.2%.

A three-fold increase was also noted in the perceived persistence of the sweetness of trehalose. (Portmann and Birch, 1995). Trehalose is one of the most chemically stable sugars (Birch, 1963; Elbein, 1974). Trehalose 1,1 glycosidic linkage makes it essentially non-reducing, highly resistant to hydrolysis, and chemically inert in its interactions with proteins

(Colaco and Roser, 1995). It is stable in a wider pH range, compared to other sugars and less soluble in water (34 g/100 g H₂O at 5 °C and 40.6–69 g/ 100 g H₂O at 20 °C) than sucrose. The melting temperature can considerably vary due to its polymorphic nature, which can exist as anhydrous or dihydrate (α , β or γ) (Kubota, 2008). Its solubility and osmotic profile is however similar to maltose. Trehalose has gained regulatory approval in many countries. It was approved in 1991 in the UK as a novel food for use as a cryoprotectant for freeze-dried foods at concentrations of up to 5%. It was then approved as a food ingredient in Korea and Taiwan in 1998 with no usage limits. In the year 2000 it was affirmed a generally recognized as safe (GRAS) status by the US FDA. JECFA reviewed and approved trehalose in June 2000 but no ADI (Acceptable Daily Intake) was specified. Regulatory approval as a novel food or food ingredient in Europe was granted in September 2001 (Richards et al., 2002).

When ingested, trehalose is enzymatically hydrolyzed in the small intestine by a trehalose-specific disaccharidase into two D-glucose molecules which are subsequently absorbed and metabolized. Disaccharides such as maltose, sucrose and lactose are digested in similar physiological processes (Dahlqvist, 1974). The disaccharidase specific for trehalose is called trehalase (Dahlqvist, 1962). The caloric density of trehalose is 4 kcal/g because it is broken down to glucose. Although there is no gender or age differences associated with trehalase activity (Welsh et al., 1978), there are reports of ethnic differences in the ability to ingest trehalose. Some section of the Asian population have been reported to have slightly lower tolerance to trehalose (Bergoz, 1971; Bolte et al., 1973; Oku and Okazaki, 1998). Despite these differences, safe human consumption of trehalose in doses up to 50 g has been demonstrated (Ushijima et al., 1995). There appear to be no barrier therefore for the inclusion of trehalose in future food products.

2.3.2.4 Isomaltulose

In the last two decades there has been an increasing interest in the use of isomaltulose. Also known as Palatinose[®] or Lylose[®], isomaltulose is found naturally in honey and sugar cane extract and has been considered a promising substitute for sucrose. It is a reducing disaccharide (6-O- α -D-glucopyranosyl-D-fructofuranose) (CAS. No. 13718-94-0) consisting of a glucose and a fructose joined by an α -1,6 glycosidic bond (figure 2.5).

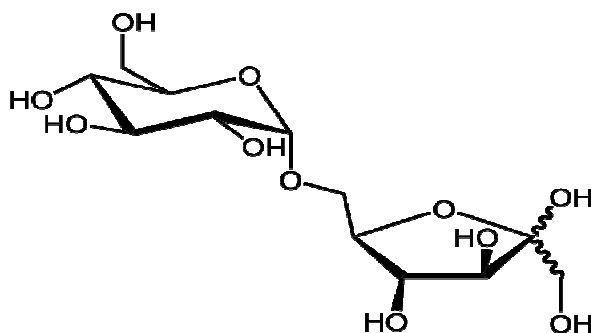


Figure 2.5 Chemical structure of isomaltulose

It is industrially produced from sucrose by enzymatic rearrangement of the glycosidic linkage from a (1,2) - fructoside to a (1,6) - fructoside, followed by crystallization (Weidenhagen & Lorenz, 1957; Mauch & Schmidt-Berg-Lorenz, 1964; Schiweck, 1980; Schiweck et al., 1990). Isomaltulose has a mild sweet taste, with about 50% the sweetness of sucrose (Takazoe et al., 1985; Hashimoto et al., 1987; Huang et al., 1998). Its sweetness profile is similar to sucrose leaving no aftertaste and when used as a sugar replacer in confectionery and chocolate no difference in sweetness was noted (Huang et al., 1998; Cheetham et al., 1982). Its naturally sweet taste and physical and organoleptic similarities to sucrose in foods and beverages make this disaccharide a popular choice in the food industry as a low calorie sweetener (Schiweck et al. 1991; Lina et al. 2002; Wu and Birch 2005). Without changes to traditional manufacturing processes, isomaltulose has been applied as a sugar replacer in bakery products, candies, canned fruits, chewing gum, chocolate-based products, confectionery, sports drinks and toothpaste (Irwin & Sträter, 1991). It melts at a lower temperature (123-124 ° C) compared to sucrose (160 - 185 ° C) and is more stable under acidic conditions. Solutions of 20% isomaltulose boiled at pH 2.0 for 1 h, did not undergo hydrolysis (Irwin and Sträter, 1991). At room temperature the solubility of isomaltulose is half that of sucrose and viscosities of aqueous solutions of both sugars are similar. It is not hygroscopic as sucrose and lactose but has thermal stability slightly lower than that of sucrose.

Being an isomer of sucrose, isomaltulose is completely metabolized in the intestine although much more slowly than sucrose and other sugars (Lina et al. 2002). This causes a very low glycemic and insulinemic response a property that is favourable for both diabetics and nondiabetics (Kawai et al. 1989). Unlike sucrose, isomaltulose is barely fermented by oral

microbes and inhibits the formation of insoluble glucans making it non-cariogenic. Several studies have shown similarities in gastrointestinal tolerance of isomaltulose and sucrose even at high dose levels. In humans no intestinal discomfort occurred at levels up to 50 g/day (Spengler and Sommerauer, 1989; Kashimura et al., 1990). Isomaltulose was designated Generally Recognized As Safe (GRAS) in 2006 by the US Food & Drug Administration (FDA) and has been granted a non-cariogenic health claim. The overall physicochemical properties of isomaltulose thus permit its use as a sucrose substitute in most sweet foods.

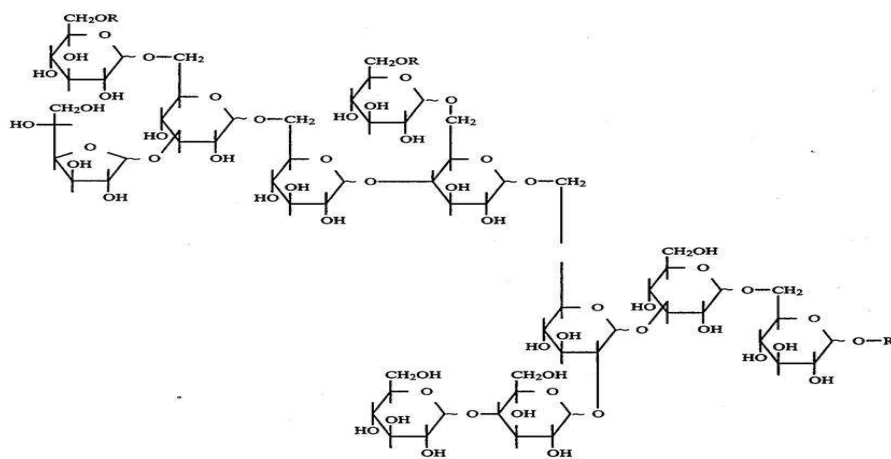
2.3.3 Low - digestible carbohydrate polymers

Fiber or fiber-like ingredients known as low-digestible carbohydrate (LDC) polymers have been utilized within the past two decades as bulking agents in the manufacture of sugar-free chocolates. They are composed of sugars such as glucose, mannose and fructose linked together in such a way that their digestibility as well as caloric contribution is significantly reduced. They come from many diverse and unique sources lending them to have many variations in their functional characteristics. These carbohydrate polymers tend to have a high molecular weight often providing viscosity and body to most food applications. They can be used to help obtain a sugar-free claim as well as fiber claim (Jamieson, 2008). LDC polymers not only provide the bulk needed to replace sucrose but are typically more slowly digested through various metabolic pathways yielding lower calories, reduced glucose response, increased satiety and a reduction in dental caries (cavities). Even though LDC polymers have been used for decades by diabetics, the landscape of ingredients available today as well as their understanding has changed greatly. This has opened the door for product developers to create sugar-free products of higher quality that look, taste and eat like traditional confections. The end results are products proving to be useful tools for consumers to enjoy while trying to live a healthier lifestyle. Polydextrose, inulin, oligofructose and maltodextrin fall in this category and are extensively discussed.

2.3.3.1 Polydextrose

Polydextrose is a randomly linked polymer of glucose with similar technological properties as

sucrose except for sweet taste (Burdock & Flamm, 1999; Afoakwa et al., 2007b; Beckett, 2009). It is regarded as either a resistant polysaccharide (RP) or a resistant oligosaccharide (RO) with an average degree of polymerization (DP) of ~12 (weight average molecular weight of ~2,000) (Craig, 2001). Polydextrose, as a commercial available preparation, is produced by the condensation of a melt which consists of approximately 89% D-glucose, 10% sorbitol and 1% citric acid on a weight basis (Colliopoulos et al., 1986). The chemical structure is shown in Figure 2.6.



R= hydrogen, glucose, sorbitol, citric acid, or polydextrose.

Figure 2.6 Chemical structure of polydextrose

Since polydextrose is a randomly bonded condensation polymer of D-glucose with some bound sorbitol and citric acid, there is no specific chemical formula for the product. It is a condensation polymer which means that whenever a glucose molecule attaches to the polymer chain, it loses a molecule of water. Therefore, the repeating unit in the polymer is not $C_6H_{12}O_6$ but $C_6H_{10}O_5$ with MW 162g/mol (Allingham, 1982). Polydextrose has a broad molecular weight range with 90% of the molecules being between 504 and 5000g/mol MW (Kibbe, 2000).

Typically offered as an amorphous powder, polydextrose is hygroscopic and can easily pick up moisture. This is a great property for controlling water activity and shelf life in certain applications but could be counterproductive in others like hard candy by increasing

stickiness and limiting shelf life. Polydextrose has been successfully incorporated into a wide range of foods including baked goods, beverages, confectionery and frozen desserts. Polydextrose provides the bulk and appropriate textural and mouthfeel qualities usually associated with sugar and fat while lacking the sweet taste and caloric value connected with those conventional food ingredients (Lauridsen, 2004). Physiological benefits of polydextrose include fermentation in the lower gastrointestinal tract, production of short-chain fatty acids (SCFA), fecal bulking, reduced transit time, and glucose homeostasis (Craig et al., 1998). The structural compactness and complexity of polydextrose prevents mammalian digestive enzymes from readily hydrolyzing the molecule, thus imparting reduced caloric content (Craig et al., 1998). When used as a sugar replacement, polydextrose is generally combined with intense sweeteners in order to provide the desired sweet taste in the product in question.

Polydextrose is approved as a direct food additive by the US Food and Drugs Administration for use as a nutrient supplement, texturizer, stabilizer or thickener, formulation aid and humectant and has an accepted caloric value of 1.0 kcal/g. EU directive 2008/100/EC however assigns a caloric value of 2 kcal/g to polydextrose. Its low caloric value according to Burdock and Flamm (1999) is due to poor digestibility and incomplete fermentation in small and large intestines. In Japan polydextrose is considered a food not a food additive and is widely used there in fiber-fortified health beverages. It is also approved under the Japanese Foods for Specified Health Use law as an ingredient for which the label claim "provides improved intestinal function" may be made. The FDA estimated the per capita individual consumption of polydextrose for currently approved uses to be 14.3 g/day or 0.24 g/kg body weight/day, based on MRCA 5-year menu census (1982-87) (DiNovi, 1992). LDC polymers are effective tools for sugar replacement but are - as their name implies - low digestible. Subsequently, as they pass mostly untouched into the lower gastrointestinal tract, they can lead to osmotic imbalances and/or fermentation by bacteria. As a result, if over-consumed, individuals may experience loose stools and gas. JECFA and the European Commission Scientific Committee for Food (EC/SCF) concluded a mean laxative threshold of polydextrose of 90 g/day (1.3 g/kg bw) or 50 g as a single dose. It is approved in over 50 countries around the world and can be labeled a fiber in Argentina, Egypt, Indonesia, Japan, Korea, Poland, and Taiwan. Specification monographs are published in the Food Chemicals Codex (FCC)

(NAS, 1996) and the FAO Compendium of Food Additive Specifications (JFECFA, 1995). Knowing the requirements of the application will therefore help the product developer to understand where this ingredient can provide the most benefit for a finished chocolate.

2.3.3.2 Inulin and oligofructose

Inulin and oligofructose belong to a class of carbohydrates known as fructans. Fructans are linear or branched fructose polymers, which are either β 2 \rightarrow 1 linked inulins or β 2 \rightarrow 6 linked levans. The main sources of inulin and oligofructose used in the food industry are chicory and Jerusalem artichoke. Table 2.8 lists common fructan sources and the fructan concentrations therein. Inulin and oligofructose are considered as functional food ingredients, resulting in better health and reduction in the risk of many diseases (Kaur & Gupta 2002; Abbasi & Farzanmehr, 2009).

The average daily consumption of inulin and oligofructose has been estimated to be 1–4 g in the United States and 3–11 g in Europe. Their energy content is only 40–50% of that of digestible carbohydrates giving them a caloric value of 1.0–2.0 Kcal/g (Kaur & Gupta, 2002). EU directive 2008/100/EC assigns a caloric value of 2 kcal/g. Inulin is comprised of fructose molecules linked together and ending with a glucose molecule to form polymers of various lengths. The chemical structure is shown in Figure 2.7.

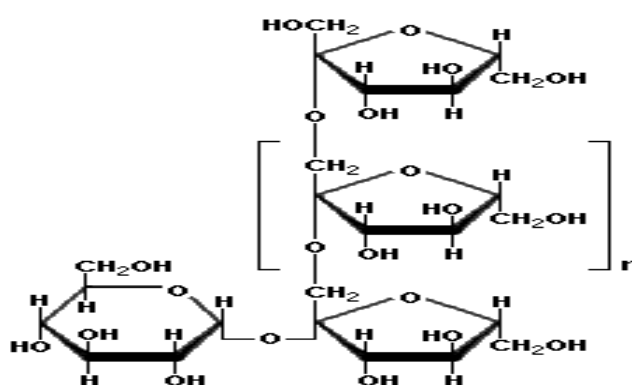


Figure 2.7 Chemical structure of inulin

Native or medium chain length inulin, as present in chicory, has a degree of polymerisation (DP) ranging from 3 to 60 monosaccharide units, with an average of about 10 (Meyer, 2011).

Inulin is processed by the food industry to produce either short chain fructans namely oligofructose (DP, 2–8; average 4) as a result of partial enzymatic (endoinulinase EC 3.2.1.7) hydrolysis or long chain fructans by applying industrial physical separation techniques (De Leenheer, 1996). Typically, the smaller the polymers, the more soluble and sweet they become.

Table 2.8 Important sources of fructans and the concentrations present on fresh weight basis (Silver, 2006)

Source	Fructan (%)
Agave	15-20
Artichoke	2-6
Asparagus root	10-15
Banana	0.3
Chicory root	15-20
Dahlia tuber	15-20
Dandelion	15-20
Edible Burdock (root)	16
Garlic	15-25
Jerusalem Artichoke	15-20
Leak	10-15
Rye	0.7
Salsify	15-20
Wheat	0.4
Yacon	15-20

The extensive use of inulin in the food industry is based on its nutritional and technological properties. For the former not only the dietary fibre properties of inulin such as the positive effect on bowel habit are important (Tungland & Meyer, 2002) but also the prebiotic properties. The technological use of inulin is based on its properties as a sugar replacer (especially in combination with high intensity sweeteners), as a fat replacer and texture modifier. When inulin is added to food in low concentrations the rheological properties and the sensory quality of the product will not be affected strongly due to its neutral or slightly sweet taste and its limited effect on viscosity (Kalyani et al., 2010). Other physico-chemical properties that are influenced by the degree of polymerization include the melting temperature and glass transition temperature, the capability for gel formation and the

subsequent gel strength and the interaction with other food components such as starch or hydrocolloids (Bishay, 1998; Zimeri & Kokini, 2003; Giannouli et al., 2004). In most cases, given all that have been discussed on the impact of functionality, it will only make up part of the sugar replacement. For example some sugar-free chocolates use inulin with other bulk sweeteners such as erythritol and isomalt. By doing so, the end result is a product that is not only of good eating quality but also well tolerated by the consumer.

Oligofructose is composed of shorter chain oligomers and possesses functional qualities similar to sugar or glucose syrups. It is more soluble than sucrose and provides about 30 to 50% of the sweetness of table sugar. Oligofructose contributes body to dairy products and humectancy to soft baked goods. It acts as a binder in nutritional or granola bars in much the same way as sugar but with the added benefits of fewer calories, fibre enrichment and other nutritional properties. Oligofructose is often used in combination with high intensity sweeteners to replace sugars providing a well-balanced sweetener profile and masking the aftertaste of aspartame or acesulfamek (Weidmann & Jager 1997). Unlike other fibers, inulin and oligofructose have no “off flavors” and may be used to add fiber without contributing viscosity. These properties allow the formulation of high fiber foods that look and taste like standard food formulations (Niness, 1999). Unfortunately inulin and oligofructose have a propensity to cause bloating and flatulence when consumed in moderate to large quantities (Brown et al., 2008). Colonic fermentation of inulin and oligofructose produces short chain carboxylic acids (acetate, butyrate and propionate), lactate and gases as products of digestion (Gibson et al., 1995).

2.3.3.3 Maltodextrin

Maltodextrin is another low digestible carbohydrate polymer that has great potential in the development of functional confectionery. Maltodextrins are defined as starch hydrolysis products with dextrose equivalent (DE) less than 20 (Dokić-Baucal et al., 2004). Dextrose equivalent is a measure of the reducing power of starch-derived polysaccharides/oligosaccharides compared with D-glucose on a dry-weight basis. The higher the DE the greater the extent of starch hydrolysis (Wang and Wang, 2000). As products of starch hydrolysis, maltodextrins contain linear amylose and branched amylopectin

degradation products and are therefore considered as D-glucose polymers joined by α -(1,4) and α -(1,6) linkages (Dokić-Baucal et al., 2004) (Figure 2.8).

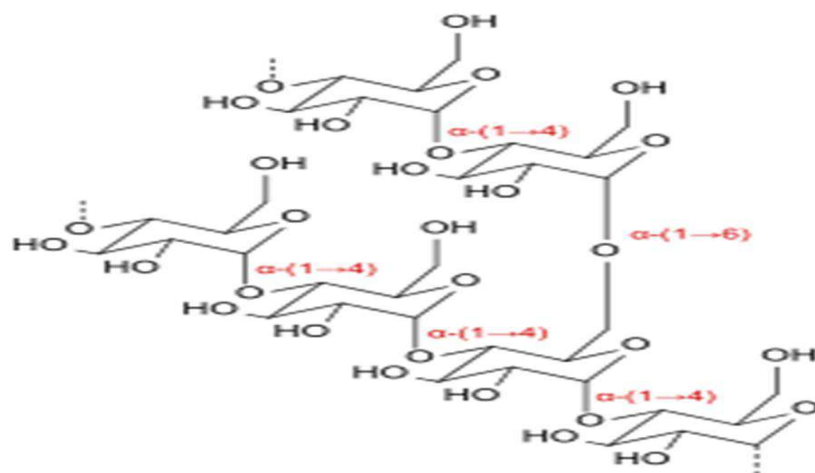


Figure 2.8 Structure of a dextran joined by α -(1,4) and α -(1,6) linkages

The type of starch (maize, oats, rice, tapioca, potato) is an important factor determining the molecular segments of maltodextrins. The ratio of linear amylose chain molecules to branched amylopectin varies depending on the source of starch (Kennedy et al., 1987; Chronakis, 1998). Maltodextrins from different botanical sources therefore exhibit different properties due to inherent differences in their chemical structures (Wang & Wang, 2000). Increase in the dextrose equivalent value results in increase in hygroscopicity, solubility and osmolality (Dokić-Baucal et al., 2004; Morris, 1984; Wang & Wang, 2000).

Maltodextrins have the ability to form weak gels which are results of interactions between helicoidal amylose fractions and branched amylopectin molecules (Chronakis, 1998). Maltodextrins have therefore been introduced in the food industry as texture modifiers, thickening agents and fat replacers (Alexander, 1995; Chronakis, 1998). It is very soluble, up to 240 g in 100g of water, but not as hygroscopic as polydextrose in powder form. It is considered GRAS by the USFDA providing 1.0-1.5 kcal/g. EU directive 2008/100/EC however assigns a caloric value of 2 kcal/g. Individuals can consume at least 60g a day over an extended period of time without any significant issues, indicating that maltodextrin can be well tolerated (Goldman, 2006).

2.3.4 Laxation and Low – digestible carbohydrate polymers

Although LDCs are effective tools for sugar replacement they are - as their name implies - low digestible. Subsequently, as they pass mostly untouched into the lower gastrointestinal tract they can lead to osmotic imbalances and/or fermentation by bacteria. As a result if over-consumed, individuals may experience loose stools and gas. In the case of fiber this is expected but it is important to realize that all other LDCs discussed here have the potential to do the same as well. And the effects can vary for each individual due to factors such as age, colonic microflora, gender, psyche, health and, of course, diet. In addition, the molecular weight and digestibility of the LDC consumed will dictate the degree of laxation that individuals experience. As a product developer, controlling the amount of low-digestible carbohydrates per serving size is important. The combination of inulin, maltodextrin and polydextrose has been found to reduce or eliminate laxative effects and/or the flatulence effects of large amounts of inulin used as bulking agent, or maltodextrin used as a bulking agent, or polydextrose used as a bulking agent, with other sugar substitutes (Goldman, 2006). Considering the thought that people may consume more than one serving in a sitting, levels above 30g of low-digestible carbohydrates per serving would not be recommended. More appropriately a target of 15g or less per serving for LDCs would be better served when formulating new products (Jamieson, 2008). Therefore, by working with the supplier to understand each ingredient's tolerance, one can provide a finished product that is enjoyable and safe for the consumer.

2.3.5 Applicability and suitability of different sweeteners and polymers in chocolate processing

Over the past decade, various researchers have investigated the use and applicability of several sweeteners and polysaccharides as bulking agents in the production of sugar-free chocolates (Bolini-Cardello et al., 1999; Golob et al., 2004; Wada et al., 2005; Melo et al., 2007; Farzanmehr & Abbasi, 2009; Shah et al., 2010; Pallazo et al., 2011). These investigations have led to various degrees of successes and challenges in their application in the modern confectionery industry.

2.3.5.1 Polyols

Maltitol has organoleptic and technological properties close to those of sucrose (Portmann & Kilcast, 1996). Its low hygroscopic character gives it the advantage of allowing the refining of chocolates under the same conditions as with sucrose and conching at temperatures up to 80°C (Olinger, 1994). It has 95% of the sweetness of sucrose, reducing the need for its combination with an intense sweetener, and has been used mainly in the manufacture of sugar-free chocolate, sweet coatings and chewing gum (Sicard & Le Bot, 1994). Isomalt has only 40% of the sweetness of sucrose, so intense sweeteners must be used along with isomalt in chocolates (Wijers & Sträter, 2001). The use of isomalt in chocolate results in a higher viscosity compared to maltitol, sucrose, and xylitol after 18h conching at 50°C but in a lower viscosity compared to xylitol when conched at 60 °C. Different conching temperatures for chocolates with different sugar alcohols have therefore been advised (Olinger, 1994).

Consumers may reject chocolate containing xylitol due to xylitol's intense cooling effect in mouth, although this can be masked by addition of other bulk sweeteners (Olinger, 1994). This cooling effect is however absent in isomalt and maltitol (Kato & Moskowitz, 2001; Wijers & Sträter, 2001). Xylitol has 95% of the sweetness of sucrose and no additional intense sweeteners may be needed (Kato & Moskowitz, 2001). Erythritol also serves as sugar substitute in confectionery compositions, in particular chocolates. It has a sweet taste and delivers considerably less calories than sucrose but its use suffers from the perception of a cooling effect and/or a burning aftertaste. When erythritol is used as a sugar substitute, the melting of chocolate in the mouth causes an unpleasant feeling of cold. U.S Pat. No. 6,875,460 tries to reduce the cooling effect of erythritol by adding hydrogenated maltodextrin. However, the observed effect is considered as a dilution effect. Other inventions have tried eliminating this cooling effect by using fibres (preferably dietary fibres and in particular, water-soluble dietary fibres) and / or sugar esters. Brown et al. (2008) stated that erythritol is commonly combined with inulin and fructo oligosaccharide (FOS) which offer a complementary positive heat of solution. There is therefore a continued need to identify and provide agents, by means of which the cooling effect of polyols can be reduced or eliminated.

Sokmen & Gunes (2006) investigated the influence of bulk sweeteners on the rheological

properties of chocolate. Sucrose was totally replaced with maltitol, isomalt or xylitol of different particle size intervals (PSI) of 38-20, 53-38 and 106-53 μ m. The chocolate samples were conched at 65 °C for 3h in a paraffin bath and all rheological properties of the chocolate samples were measured using a shear-controlled rheometer with a concentric cylinder system. The Herschel-Bulkley model fitted the data (viscosity, yield stress, flow behavior index) more appropriately although Casson model is widely used and recommended by IOCCC to describe the flow behavior of chocolate. Chocolates made with xylitol and maltitol resulted in a similar plastic viscosity as the reference chocolate made with sucrose. The plastic viscosity of chocolate with isomalt was significantly higher and the difference was more apparent at lower particle sizes. Sokmen and Gunes (2006) associated this with isomalt's higher solid volume fraction in chocolate because the density of isomalt was 1.50 g/cm³, slightly lower than the other sugar alcohols, 1.63 and 1.52 g/cm³ for maltitol and xylitol, respectively. This implies that chocolate with isomalt had more solids and a larger surface area since all sweeteners were added to the chocolate mix on a weight basis. The higher plastic viscosity caused by isomalt may also be associated with its other physical properties such as specific surface area, crystallinity and hygroscopicity that were not evaluated in their study. The yield stress of chocolate with maltitol was significantly higher than chocolate with isomalt. The authors associated this with maltitols PSD which contained higher amounts of smaller particles out of range than the other sucrose substitutes. The yield stress also decreased significantly with increase in particle size with interactions between particle size interval (PSI) and bulk sweetener type being significant ($P=0.001$).

Overall, chocolates with xylitol recorded the highest flow behavior index. The flow behavior index also increased with decrease in particle size. The results of the apparent viscosity were in agreement with that of the plastic viscosity with isomalt chocolate recording higher value than sucrose and maltitol chocolates. The effect of bulk sweeteners on apparent viscosity was more apparent with finer particles. As particle sizes decreased, the apparent viscosity increased substantially. The authors concluded that large particle sizes result in better rheological properties for manufacturing processes but may adversely affect sensory properties. A better control of PSD of bulk sweeteners, chocolate mix and conching conditions is therefore needful to determine the effects of bulk sweeteners on physical and

sensory properties. Consequently, addition of bulk sweeteners on volumetric basis may reflect their effect on rheological properties more accurately.

2.3.5.2 Intense sweeteners

The time-intensity method has become a useful tool in the comparison of the perception of sucrose sweetness over time with that of other sweeteners. Melo et al. (2007) applied the time-intensity analysis in the sweetness perception of diabetic milk chocolates. They concluded that chocolates sweetened with sucrose and sucralose presented similar results with regard to sweetness profile. This was, however, not the case for chocolates sweetened with stevioside (Melo et al., 2007). Palazzo et al. (2011) determined isosweetness concentrations of sucralose, rebaudioside and neotame as sucrose substitutes in new diet chocolate formulations using the time-intensity method. Sucrose was replaced by polydextrose and erythritol as bulking agents together with the above mentioned sweeteners. Sucralose presented the best result as compared with the traditional sample containing sucrose. The sweetness of rebaudioside decreased with increase in concentration. Neotame as a sweetener presented less satisfactory replacement in milk chocolates. The authors therefore stressed the importance of studying each sweetener in foods they could be used in because their sweetness potencies depend on the dispersion matrices in which they are found.

2.3.5.3 Carbohydrate polymers

Processing conditions have been known to have great effect on sugar substituted chocolates. Zumbé (1992) mentioned that, in view of the use of polydextrose as bulking agents in sugar-free chocolates, the temperature during conching should be kept below that at which the water of crystallization inherently present in these ingredients is released. This avoids any undesirable increase in viscosity or agglomeration of the mixture. Some sugar-free chocolates use inulin with other bulk sweeteners such as erythritol and isomalt resulting in products of good eating quality and well tolerated by consumers. Golob et al. (2004) studied the influence of inulin and fructose on the sensory characteristics of chocolate and found that sucrose replacement with inulin in milk chocolate formulation did not result in perceived sensory differences compared to the control by a consumer panel. The most common functional benefits of inulin in chocolate include modulation of the cooling effect

during melting in the mouth and improvement of the chocolate flavour. A major obstacle to the use of inulin as bulking agent however is the presence of various amounts of glucose and fructose which is naturally contained therein making it difficult to dry, handle and store. When such inulin products are manipulated in the mouth, a sticky hard substance is formed caused by the insolubility at body temperature in the saliva (Berghofer, 1993).

Farzanmehr and Abbasi (2009) substituted sucrose with different levels (0-100%) of inulin, polydextrose, and maltodextrin as bulking agents in prebiotic milk chocolate formulations. The Casson model showed the best fitting for predicting rheological properties and all chocolates showed thixotropic and shear thinning behaviors. Chocolate formulations containing high levels of sugar substitutes had higher moisture content, Casson viscosity and yield stress than the control sample made with sucrose. In contrast, the lowest moisture content, Casson viscosity and yield stress, were observed for chocolates with moderate amounts of sugar substitutes. In the physical analyses, formulations with high ratios of polydextrose and maltodextrin were more moist and softer than the control. Lowest moisture content and highest hardness were observed when moderate ratios of polydextrose and maltodextrin were used. Farzanmehr and Abbasi (2009) attributed this to the higher hygroscopicity of maltodextrin and polydextrose. In contrast, inulin due to its low hygroscopicity did only influence the moisture content at very high levels. Chocolate formulation with ratios of 50:25:25% for inulin, polydextrose and maltodextrin, respectively, was the hardest chocolate. Hardness of chocolates formulated with 100% inulin was similar to the control sample. In the sensory analysis, chocolate formulations with high ratios of maltodextrin were very sticky and, after consumption, created a short thin-layer film on the surface of the tongue and mouth hole. This probably accounted for the low melting rate, mouth coating and overall acceptability scores recorded for formulations with high ratios of maltodextrin. Melting rate score however increased with increasing inulin and polydextrose contents and reached its highest values at the highest levels of inulin and polydextrose. Similar trends were observed regarding mouth coating and overall acceptability (Farzanmehr & Abbasi, 2009). The authors concluded that, the type and ratio of sugar substitutes induced various effects on physicochemical, textural and sensory properties of low-calorie milk chocolate. Higher inulin and polydextrose and lower proportions of maltodextrin greatly improved sensory attributes of the milk chocolates. Inulin, polydextrose and maltodextrin

concentrations of 14-32% and 71-84%, 7-26% and 67-77%, and 0-20% respectively, were stated as the optimum applicable range for the sugar substitutes. This indicates that inulin and polydextrose can be used in various ratios and owing to their noticeable effects can improve chocolate properties even at very low ratios, whereas maltodextrin should only be added at low ratios (<20%) (Farzanmehr & Abbasi, 2009).

Shah et al. (2010) replaced sucrose with inulin (HP, HPX and GR) with different degrees of polymerization and polydextrose as bulking agents together with the intense sweetener stevia in the development of sugar-free chocolates. Inulin HP (average DP \geq 23, long chain inulin), Inulin HPX (average DP \geq 23, long chain inulin with low solubility) and Inulin GR (average DP \geq 10) were used. Replacement of sucrose by the above ingredients resulted in darker chocolates. Shah et al. (2010) attributed the differences in colour (L^* values) to changes in surface properties, mainly roughness, of chocolate caused by the sugar substitutes since processing conditions were the same for all samples. Smoother surfaces always provide for lighter colours of chocolate products (Briones et al., 2006). The melting point temperature of all chocolate samples ranged from 30.8 °C to 32.6 °C with the control sample and milk chocolates with inulin HP having significantly higher melting temperature compared to the other samples. The authors gave two explanations to the differences in melting temperature. Firstly, the fat in chocolates made with sucrose and inulin HP are in the form of V B₂ triple chained crystals, the most stable form of cocoa butter, produced in a well tempered chocolate. The second possibility is the effect of inulin and its average degree of polymerization. Increase in melting point with increasing average degree of polymerization of inulin has been reported by Blecker et al. (2003). Hébert et al. (1998) also suggested the occurrence of two crystal populations differentiated by crystalline thicknesses, and with thicker crystals having a higher melting point, as the reason for complex melting behavior of inulin.

Replacement of sucrose with inulin and polydextrose as bulking agents had no substantial effect on chocolate hardness except for chocolates made with inulin HPX which was less hard than the other samples. Several factors including composition, manufacturing conditions and tempering and consequently fat crystal polymorphism would influence the final hardness of chocolate (Afoakwa, 2010; Beckett, 2008). Shah et al. (2010) explained the hardness behavior of inulin HPX as likely to be the result of poor tempering since the authors

later found that chocolates made with inulin HPX had a melting point of 30.8 °C. Hardness is a useful indicator of good tempering or the degree to which a fat crystal network has been formed. The authors therefore recommended the modification of the standard operating tempering procedure for inulin HPX since all samples were tempered using the same procedure. In the rheological analysis, the Herschel-Bulkley model showed the best fitting for predicting rheological properties. Chocolates with inulin HPX and HP exhibited higher plastic viscosity than the control. The plastic viscosity of chocolate with inulin GR was however lower than the control. The plastic viscosity thus increased with increase in degree of polymerisation of inulin. The authors associated the higher plastic viscosity of chocolates made with inulin HPX and HP to their higher solid volume fraction in chocolate because the density of inulin HPX (470 g L⁻¹) and HP (490 g L⁻¹) were slightly lower than that of inulin GR (580 g L⁻¹). The yield stress of chocolate with inulin HPX was slightly higher than the control while the other samples were slightly lower.

Flow behavior is very important in determining the stability of chocolate products. Overall, sucrose replacement with inulin HPX or HP resulted in a higher flow behaviour index than sucrose replacement with inulin GR. This could be due to the fact that the consistency coefficient of chocolate with inulin HPX and inulin HP decreased slightly as the shearing time increased and as a result, flow behavior index increased. Another possibility is that, presence of more crystals in the chocolate with inulin HPX and inulin HP could have caused difficulty in crystal alignment during the chocolate manufacturing process resulting in a slight increase in flow behavior index (Briggs & Wang, 2004). The viscoelastic behavior of chocolate is directly related to the cooling rate of chocolate as fat in chocolate solidifies in a specific way. Replacement of sucrose with stevia as a sweetening agent and inulin and polydextrose as bulking agents had no major impact on elastic behavior of chocolate mixes during the initial stages of tempering. More evident effects were observed during the second cooling stage below 20 °C and were apparently affected by the degree of polymerization of inulin. Addition of inulins with lower degree of polymerization resulted in lower elasticity of solidified chocolate whereas inulin HP had a similar elastic behavior in comparison to that of the control. Due to the effect of temperature on inulin solubility, the lower viscoelasticity observed in the samples may be due to interference of more soluble (short chain) inulins with fat crystallization. In the sensory analysis with untrained consumer panel, panelists

preferred the control chocolate over the sucrose-free types but their next preference was chocolate containing inulin with the highest DP. Sucrose replacement with inulin significantly lowered the smoothness acceptability and mouthfeel. Flavor/taste acceptability decreased with decrease in inulin DP. Shah et al. (2010), as part of their conclusions, recommended inulin HP (high DP) as suitable for sucrose free chocolate formulations since chocolate with inulin HP in combination with stevia and polydextrose resulted in very similar physico-chemical and sensory characteristics in comparison to sucrose sweetened milk chocolate. Inulin addition to sucrose-free chocolate formulations had no major effects on particle size, melting point and composition. Inulin HPX and GR, due to their shorter chain length in comparison with inulin HP, did not result in the same physico-chemical, rheological and sensory properties as inulin HP.

Conclusions

The development of high-quality sugar-free chocolate requires the use of the most appropriate ingredients that could completely replace sugar without negatively affecting the rheological, physical and sensory properties. In chocolates, sucrose is utilized as a bulking agent, texture modifier, mouthfeel modifier, flavor enhancer and preservative, in addition to providing sweetness. Sucrose substitution with intense sweeteners such as saccharin, acesulfame- K, sucralose, stevioside or thaumatin, and with bulking agents such as polydextrose, maltodextrin and inulin, have great potential for the successful manufacture of sugar-free chocolate products with the desired quality of their sugar counterparts. The characteristics of the major types of intense and bulk sweeteners have been reviewed. Understanding the properties of these ingredients would lead to the development of sugar-free chocolates that meet consumers expectations.

Chapter 3: Optimization of processing conditions and rheological properties using Stephan mixer as conche in small-scale chocolate processing

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3.1 Introduction

Conching is an important stage in chocolate processing. During this stage, heterogeneous, flaky, dry refined chocolate paste is turned into a free-flowing suspension of molten chocolate resulting in the removal of moisture, acids and undesirable flavours, and consequently leading to the development of the final chocolate flavour and texture (Beckett, 1999; Afoakwa, 2010). The conching process is named after the containers that were originally used, which resembled shells; *concha* means “shell” in Spanish. The function of the conche was initially attributed to reduce the particle size and to guarantee the fluidity of the chocolate mass. However, after the development of refiner machines this function started to be secondary, and then flavour modification was credited to the conching process (Beckett, 1994; Awuah, 2002).

Conching is normally carried out by agitating chocolate at $> 50\text{ }^{\circ}\text{C}$ for some hours (Beckett, 2000). The conching process can last for as little as four to six hours to few (1-3) days. Generally, a two-stage process is used. The first stage converts flake or powder into paste by mechanical or heat energy, driving off moisture and undesirable volatiles, effects oxidations and distributes lipids through a continuous fat phase. The second stage converts the thick paste into a free flowing liquid through addition of cocoa butter and lecithin (Chevalley, 1999; Afoakwa et al., 2007). Conching conditions show interactions between time and temperature so that higher temperatures reduce processing time. The length of time required varies, depending on the origin of the chocolate being conched, and any ingredients conched with it. Dark chocolates are typically conched at higher temperatures, $70\text{ }^{\circ}\text{C}$ or up to $82\text{ }^{\circ}\text{C}$ (Minifie, 1989; Awuah, 2002; Afoakwa et al., 2009). A conche can be relatively small for retail establishments or have a large tank suitable for large-scale production facilities. The many types of chocolate conching machines allow for the diverse array of many chocolate producers that are found on the market. Conditions may be modified (generally shortened) by pre-treatment of chocolate liquors as thin films at temperatures $> 100\text{ }^{\circ}\text{C}$ (Minifie, 1989; Afoakwa et al., 2007; Afoakwa, 2010). Conching machines come in various styles and many offer low-maintenance, optimal heat transfer, and high-strength seals to ensure a cost-effective means of production. A compromise of the most force and power versus a low risk of overheating chocolate is also preferred. The chocolate is kept warm and liquid through the friction created by the conching machine,

which grinds the chocolate against a hard surface using rollers (<http://www.wisegeek.org/what-is-conching.htm>).

Chocolate is processed in the molten state and its rheology is therefore of direct significance to manufacturing and product quality (Taylor et al., 2009). The flow behavior of liquid chocolate is influenced by processing (refining, conching, tempering) as well as formulation (particle size distribution, amount of fat, amount and type of emulsifiers) (Vavreck, 2004; Schantz & Rohm, 2005; Afoakwa et al., 2007). Rheological properties not only determine the efficiency of processes involving mixing and pumping, but also play a crucial role in chocolate applications such as enrobing, shell formation and moulding processes (Servais et al., 2004). As such, controlling the rheological properties of chocolate is paramount. To give chocolate a suitable viscosity, additional cocoa butter and lecithin can be added towards the end of conching to thin chocolate prior to tempering. The solid particles such as sugar, non-fat cocoa and milk powder (depending on type) are coated with fat which promotes the smooth texture and snap desired in chocolate (Prawira and Barringer, 2009). Imperfections in the conching process may result in inadequate distribution of the fat on solid particles generating a heterogeneous chocolate, migration of fat and sugar, acid flavour, and absence of desirable flavours (Cidell & Alberts, 2006).

Chocolate rheology is quantified using two parameters [yield stress and apparent (plastic) viscosity] with relevance to manufacture (ICA, 2000; Chevalley, 2008). Yield stress is a material property denoting transition between pseudo-solid and pseudo-liquid behaviours related to minimum shear stress at first evidence of flow, or transition from elastic to viscous deformation (Doraiswamy et al., 1991; Yoo et al., 1994). Plastic viscosity determines pumping characteristics, filling of rough surfaces, coating properties and sensory character of chocolate mass (Seguine, 1988).

3.2 Research strategy

Available conches require raw materials between 5 kg (laboratory scale) to multiple tonnes (industrial scale) and the conching process lasts for as little as 4 to 6 hours to few (1-3) days. This process results in high raw material usage and causes delays in chocolate processing even when small quantities of finished chocolate is required for chocolate-based production

applications such as panning, enrobing and extrusion. As many applications for chocolate production (enrobing, panning, extrusion, moulding) require careful control of the flow properties of the molten chocolate produced in smaller batches which can only be achieved through conching, this study was aimed at investigating the suitability of using Stephan mixer as a conche in small scale chocolate processing. Attainment of the optimum processing conditions for the use of Stephan mixer as conche to yield similar flow (rheological) properties as a reference Buhler Elk'Oline conche would enhance our knowledge and understanding on the optimum use of raw materials and processing times during small scale chocolate productions. This will have significance for reducing waste of processing materials and conching times with assurances in quality and shelf characteristics of products.

3.3 Materials and methods

3.3.1 Raw materials

Cocoa liquor and cocoa butter of Ghanaian origin were obtained from Cargill (Mouscron, Belgium). Sucrose (pre-broken) was supplied by Barry Callebaut (Wieze, Belgium). Soy lecithin (containing 62% acetone-insoluble matter) was supplied by Soya International (Roslyn, USA).

3.3.2 Sample preparation using the Stephan mixer

Chocolate was formulated with 0.4% w/w lecithin, 11.6% w/w cocoa butter, 40% w/w cocoa liquor and 48% w/w sucrose. The ingredients were mixed in a Vema mixer (Vema BM 30/20, Vemaconstruct, NV Machinery Verhoest, Izegem, Belgium) and refined using a 3-roll refiner (Exakt SOS Apperatebau GmbH & Co. KG, Norderstedt, Germany) to 28-30 μm particle sizes. The Stephan mixer that was used for the conching process is shown in Figure 3.1. It comprises a jacketed pan, a rotary blade for mixing and a system control unit (for time and speed control), connected to a temperature controlled water bath. Seven hundred (700) grams of refined chocolate was placed in the Stephan Mixer (Stephan Universal Machine UMC 5, STEPHAN food service equipment GmbH, Hameln, Germany) and conched by varying the time, temperature, and blade rotational speed for both dry and wet conching

procedures as done with the Buhler Elk'Oline conche. The dry conching temperature was kept constant at 65 °C but with varying time (10 min and 15 min) and blade rotary speed (750 rpm, 1500 rpm, 2250 rpm and 3000 rpm).



Figure 3.1 Stephan Universal Machine UMC5

Lecithin and cocoa butter were added after dry conching and this was followed by wet conching at varying temperatures (45 °C and 50 °C), times (10 min and 15 min) and rotary speeds (750 rpm, 1500 rpm, 2250 rpm and 3000 rpm). The resulting molten chocolate samples obtained were kept in sealed plastic containers at ambient temperature (20-22 °C) for analysis on their flow (rheological) properties (Casson yield stress, Casson viscosity and thixotropy). Typical images for the processes are shown in Figure 3.2 (a-c).

3.3.3 Sample preparation using Buhler Elk'Oline conche

Chocolate was formulated, mixed and refined as outlined in Section 3.3.2. Conching was however done using the Buhler Elk'Oline conche (Richard Frisse GmbH Bad Salzuffen, Germany) instead of the Stephan mixer. 4 kg of refined chocolate was dry conched by clockwise mixing at 1200 rpm, at 60 °C for 120 min, followed by anticlockwise shearing at 1200 rpm at 70 °C for 240 min. Lecithin and cocoa butter were then added after the dry conching and continued with wet conching for effective mixing and liquefaction. This was achieved by clockwise mixing of 2400 rpm at 45 °C for 15 min, followed by anticlockwise

shearing of 2400 rpm at 45 °C for 15min. The molten chocolate samples obtained were kept in sealed plastic containers at ambient temperature (20-22 °C) for analysis on their flow (rheological) properties (Casson yield stress, Casson viscosity and thixotropy). Chocolate samples prepared with the Buhler Elk'Oline conche was used as the reference.

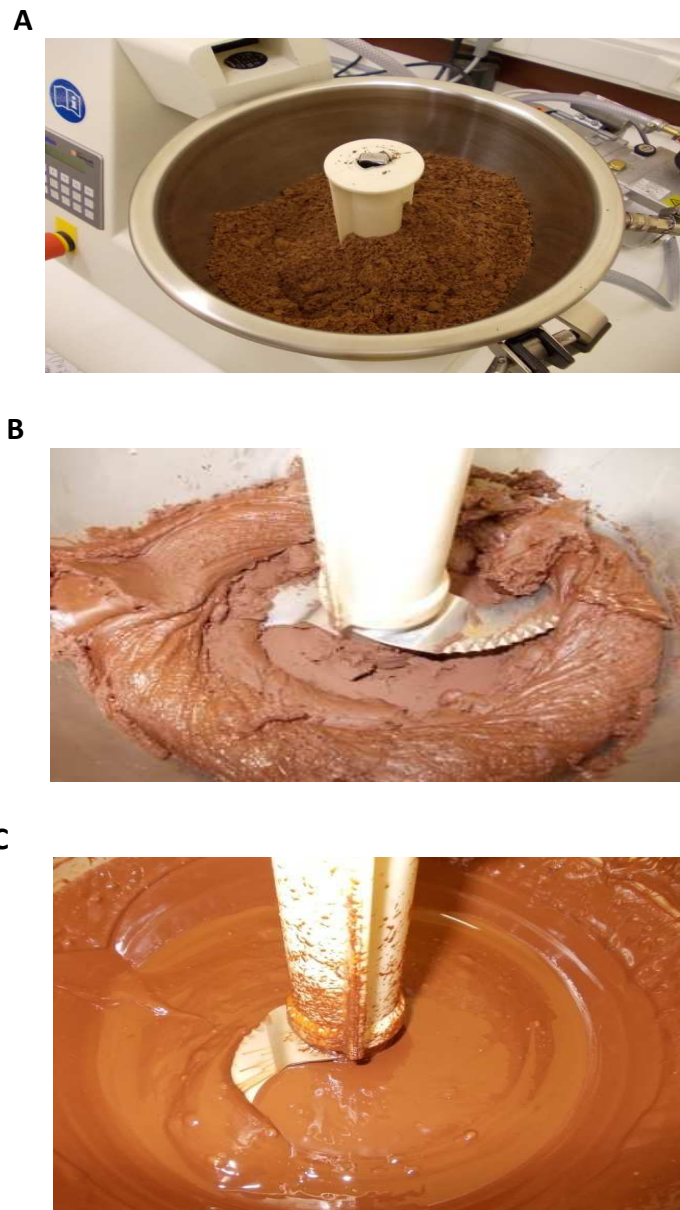


Figure 3.2 Typical images of chocolate samples at different stages of the conching process with the Stephan mixer (A) refined chocolate before conching (B) chocolate paste after dry conching (C) final molten chocolate after wet conching.



Figure 3.3 Buhler Elk'Oline Conche

3.3.4 Analytical methods

Rheological properties of the molten chocolates were studied using an AR2000ex shear rate-controlled rheometer (TA Instruments, New Castle, Delaware, USA) with concentric cylinder system (cup and bob). Chocolates samples were prepared by heating in an oven at 52 °C for an hour for melting. Approximately 20 g of molten chocolate samples were weighed into the cup and measurements were performed using the ICA (2000) official method for chocolate. Samples were pre-sheared at 5 s⁻¹ at 40 °C for 5 min before starting the measurement cycle. Shear stress was measured as a function of increasing shear rate from 2 s⁻¹ to 50 s⁻¹ (ramp up), holding at 50 s⁻¹ for 60 sec, then decreasing from 50 s⁻¹ to 2 s⁻¹ (ramp down). The data were fitted to the Casson model (Eqn. 1.2) and the Casson yield stress, Casson viscosity and thixotropy were deduced from the results. The difference between yield stresses measured at a shear of 5 s⁻¹ during ramp up and down in shear was used to represent thixotropy. All treatments and analyses were conducted in triplicates and the mean values and standard deviations recorded.

3.3.5 Statistical analysis

The three key experimental variables were dry conching time, wet conching temperature and wet conching time. A 2 x 2 x 2 factorial experimental design was used with principal factors: Dry conching time: 10, 50 min; Wet conching temperature 45, 50 °C; Wet conching

time 10, 15 min. Statgraphics Centurion XV (Graphics Software System, STCC, Inc, Rockville, USA) was used to examine the rheological properties (Casson viscosity, Casson yield stress and thixotropy) using analysis of variance (ANOVA) and multiple range tests to determine effects of process variables and their interactions. Tukey's honestly significant difference tests were carried out to determine differences ($P \leq 0.05$) between factor levels.

3.4 Results and discussion

3.4.1 Effect of Conching time and temperature

The flow properties of the molten chocolate samples were characterized using the Casson model (Eqn 1.2). The Casson model has often been successfully applied to analyze the rheological properties of chocolates (Keogh et al., 2003; Briggs and Wang, 2004; Afoakwa, 2010). The Casson yield stress is the force required to initiate flow in molten chocolate. It represents the low shear-rate properties of chocolate and is affected by particle–particle interaction, the amount and specific surface area of the particles, emulsifiers, and moisture (Servais et al., 2004; Afoakwa et al., 2007). A major contribution to the Casson yield stress is the absolute distance between solid particles in the chocolate (Afoakwa et al., 2007). The Casson yield values from this work were within the range reported for dark chocolate, i.e. 4–32 Pa (Aeschlimann & Beckett, 2000). The results are as shown in Table 3.1.

Chocolate samples dry conched for 10 min followed by wet conching at 45 °C for 10 min recorded the highest Casson yield stress of 10.41 Pa while samples dry conched for 15 min followed by wet conching at 50 °C for 15 min recorded the least Casson yield stress of 7.71 Pa. The longer time of dry conching coupled with the high time - temperature combination of 50 °C for 15 min for wet conching could have resulted in most particles been coated with cocoa butter, hence, decreasing particle-particle interactions. This resulted in lowering the minimum stress required to initiate flow, hence, the lower Casson yield stress. The dry conching time and wet conching time had significant effects ($p < 0.05$) on the Casson yield stress with significant interactions ($P < 0.05$) among all three factors (Table 3.2). Casson yield stress values for samples dry conched for 10 min followed by wet conching at 50 °C and 45 °C for 15 min and 10 min, respectively, were not significantly ($P > 0.05$) different from the

Table 3.1 Effect of dry and wet conching conditions on flow properties of chocolates conched using the Stephan Mixer

Dry Conching		Wet Conching		Casson yield stress (Pa)	Casson Viscosity (Pa.s)	Thixotropy (Pa)
Temp. (°C)	Time (min)	Temp. (°C)	Time (min)			
65	10	50	10	8.77 ± 0.18 ^{bc}	2.06 ± 0.09 ^{bc}	0.68± 0.18 ^c
			15	9.45 ± 0.26 ^{cd}	2.14 ± 0.06 ^{cd}	0.80 ± 0.25 ^c
		45	10	10.41± 0.77 ^e	2.14 ± 0.03 ^{cd}	1.23 ± 0.21 ^d
			15	8.73 ± 0.45 ^{bc}	2.16 ± 0.03 ^{cd}	0.80± 0.29 ^c
	15	50	10	8.67 ± 0.47 ^{bc}	1.92 ± 0.06 ^a	0.59± 0.16 ^{bc}
			15	7.71 ± 0.41 ^a	1.98 ± 0.02 ^{ab}	0.27 ± 0.21 ^{ab}
		45	10	8.02 ± 0.20 ^{ab}	2.01 ± 0.02 ^{ab}	0.60 ± 0.19 ^{bc}
			15	8.38 ± 0.38 ^{ab}	1.90 ± 0.07 ^a	0.75 ± 0.12 ^c
Reference sample				9.86 ± 0.21 ^{de}	2.17 ± 0.09 ^d	0.16 ± 0.11 ^a

Mean \pm standard deviations of six repetitions [Means within same column with different letters are significantly different ($P \leq 0.05$).

Table 3.2 ANOVA Summary of F-Ratios showing the rheological properties

Process Variables	Casson yield stress	Casson viscosity	Thixotropy
Main effects			
A : Dry conching time	74.15*	86.96*	30.38*
B : Wet conching temp	3.27	2.72	22.68*
C : Wet conching time	9.42*	0.70	4.11*
Interactions			
A X B	2.62	1.54	0.09
A X C	0.54	5.41*	0.27
B X C	4.11*	10.72*	0.27
A X B X C	47.40*	1.66	20.37*

*Significant F-Ratios at $P \leq 0.05$

reference sample (Table 3.1). Samples dry conched for 10 min also recorded higher thixotropy values than samples dry conched for 15 min. Thixotropy is exhibited in chocolates when apparent viscosity or shear stress decreases with time of shear at a constant rate, and relates to degree of conching as well-conched chocolate should not be thixotropic (Chhabra, 2007; Beckett, 2000). During shearing, the continuous decrease in apparent viscosity and subsequent recovery of shear stress or apparent viscosity when flow is discontinued creates a hysteresis loop. In this work, the difference between yield stresses measured at a shear of 5 s^{-1} during ramp up and down in shear was used to represent thixotropy. Thixotropy values lower than 1 Pa is a good indicator of well conched chocolate (Afoakwa et al., 2007). All samples conched with the Stephan mixer showed thixotropy values below this reference point except for the sample that was dry conched for 10 min followed by wet conching at 45°C for 10 min. The chocolate sample with the least thixotropy (0.27 Pa) was the sample that was dry conched for 15 min followed by wet conching at 50°C for 15 min. This can be attributed to the longer dry and wet conching times of 15 min which could have resulted in a much better mixing of the chocolate suspension. ANOVA showed that all three processing variables (dry conching time, wet conching temperature and wet conching time) have significant ($p < 0.05$) effects on chocolate thixotropy with significant ($p < 0.05$) interactions among all three factors (Table 3.2). With the exception of samples dry conched for 15 min followed by wet conching at 50°C for 15 min, all samples exhibited significantly ($p < 0.05$) higher thixotropy than the reference sample (Table 3.1).

Casson viscosity relates to pumping characteristics, filling of rough surfaces, coating properties and sensory character of body (Seguine, 1988). Reference values between 2.1 and

3.9 Pa.s have been reported by Aeschlimann and Beckett (2000) for dark chocolates. All chocolate samples conched with the Stephan mixer fell within the range reported (Table 3.1). This means that all chocolate formulations in this study can be employed for enrobing or coating with requirements for smoother and thinner chocolates. Chocolate samples dry conched for 10 min resulted in a higher Casson viscosity than samples dry conched for 15 min regardless of the wet conching temperature. Afoakwa et al. (2007) explained that fat content and PSD (Particle size distribution) had greatest effects on plastic viscosity of dark chocolates. Since all samples had the same fat content, the distribution of the solid particles in the samples could have accounted for the high Casson viscosity of samples dry conched for 10 min compared to samples dry conched for 15 min. Particle-particle interactions for the samples dry conched for 10 min could have been higher, thereby limiting chocolate flow. When distribution of particle sizes becomes wider with a large specific surface area during dry conching, the surfaces of the particles are coated with cocoa butter, and thus reducing viscosity of suspension for any given solid concentration (Afoakwa et al., 2007). Dry conching time had a significant ($p < 0.05$) effect on the Casson viscosity with significant interactions between the dry conching time and wet conching time, and wet conching temperature and wet conching time (Table 3.2). The Casson viscosity values for all chocolate samples dry conched for 15 min were significantly ($p < 0.05$) lower than the reference sample (Table 3.1).

Overall, two samples were not significantly ($p > 0.05$) different from the reference sample for both Casson yield stress and Casson viscosity. These are the samples that were dry conched for 10 min followed by wet conching at 50 °C for 15 min and the samples dry conched for 10 min followed by wet conching at 45 °C for 10 min. The thixotropy for the latter sample was however significantly ($P < 0.05$) higher than the former. Although the sample dry conched for 15 min followed by wet conching at 50 °C for 15 min recorded the lowest thixotropy, its Casson yield stress and Casson viscosity were significantly ($P < 0.05$) lower than the reference sample.

All experiments were conducted with the blade of the Stephan mixer fixed at a rotary speed of 1500 rpm (representing 50% of the maximum blade rotary speed of 3000 rpm) for both wet and dry conching procedures. As the blade speed of the Stephan mixer is adjustable, the effect of the rotary speed of the blade on the flow properties of the chocolate was investigated.

3.4.2 Effect of blade rotary speed

The blades of the stephan mixer operate at a maximum speed of 3000 rpm. The effects of different rotary speeds of 750 rpm (25% of maximum rotary speed), 1500 rpm, (50% of maximum rotary speed), 2250 rpm (75% of maximum rotary speed) and 3000 rpm (100% maximum rotary speed) for both dry and wet conching procedures were investigated. Maintaining a dry conching temperature of 65 °C for 10 min followed by a wet conching temperature of 50 °C for 15 min, the rotary speed of the blade was varied to see their effects on the flow properties of the molten chocolate.

3.4.2.1 Constant rotary speed for dry and wet conching

Figure 3.4 shows the effect of constant blade rotary speed for both dry and wet conching. Increasing the blade rotary speed from 750 to 3000 rpm resulted in a significant ($P < 0.05$) decrease in the Casson viscosity, Casson yield stress and thixotropy (Fig. 3.4).

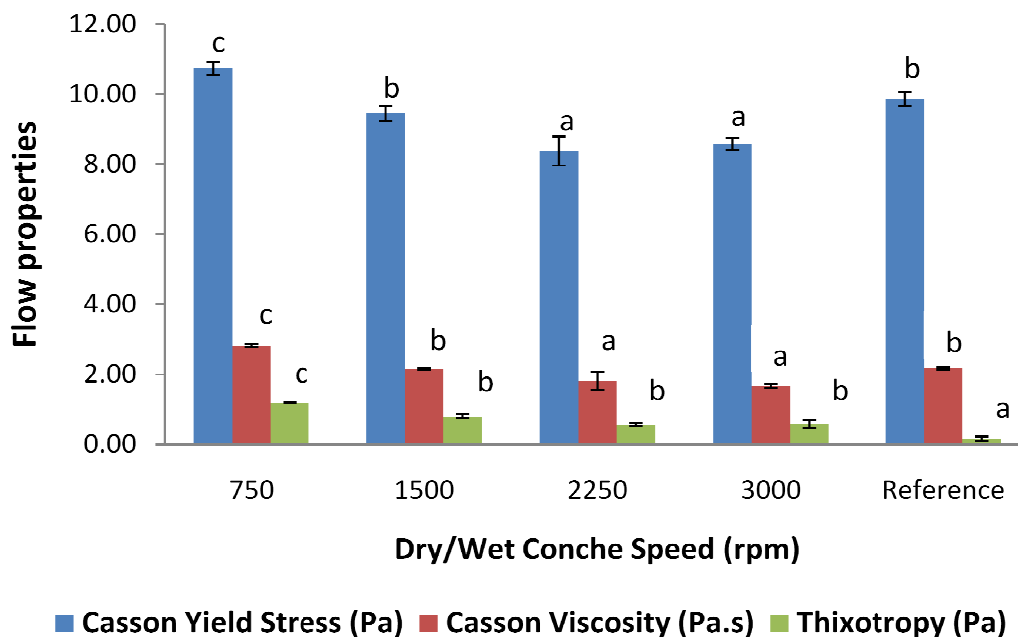


Figure 3.4 Effect of constant blade speed for both dry and wet conching on chocolate flow properties during conching with Stephan Mixer [Bars representing a particular flow property, with different letters are significantly ($P \leq 0.05$) different]

Conching at a blade rotary speed of 750 rpm resulted in higher values for all chocolate flow properties. The Casson yield stress and Casson viscosity for samples conched at a rotary speed of 1500 rpm were not significantly ($P > 0.05$) different from the reference sample. The

thixotropy values for all samples were however higher than the reference. Photographic images of chocolate samples taken after dry conching at rotary speeds of 750, 1500, 2250 and 3000 rpm are shown in Figure 3.5(A-D).

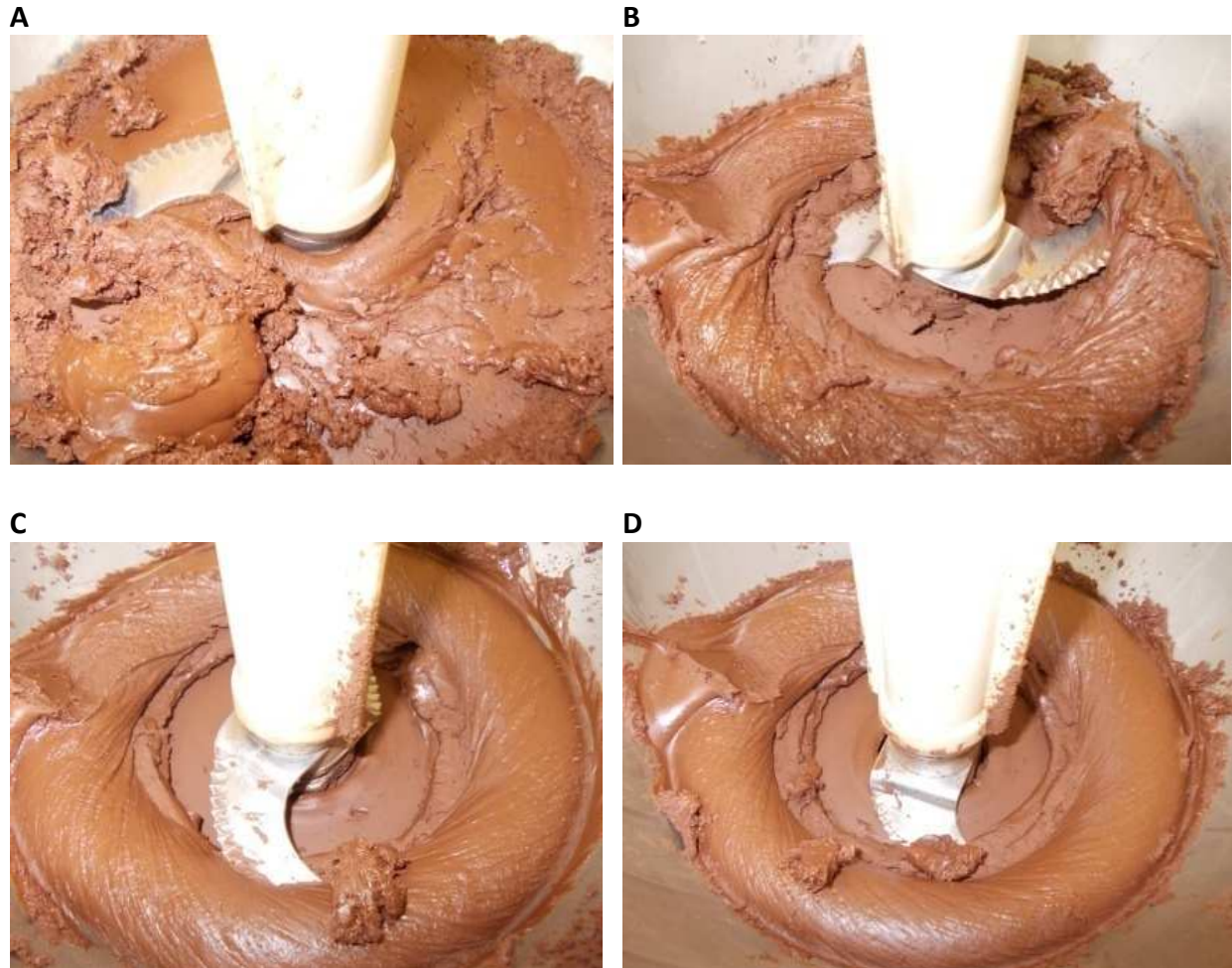


Figure 3.5 Photographic images of chocolate samples taken after dry conching at different rotary speed (A) rotary speed 750 rpm (B) rotary speed 1500 rpm (C) rotary speed 2250 rpm (D) rotary speed 3000 rpm

Samples dry conched at 750 rpm as shown, did not have a consistent mixture due to inadequate mixing whereas samples dry conched at the maximum speed of 3000 rpm showed a very homogeneous smoother chocolate (Fig 3.5D). This could explain the high Casson viscosity, yield stress and thixotropy of samples conched at the lowest speed since all particles were probably not coated with fat, limiting flow and consistency. The significantly high thixotropy for samples conched at the lowest speed can be attributed to the crowding

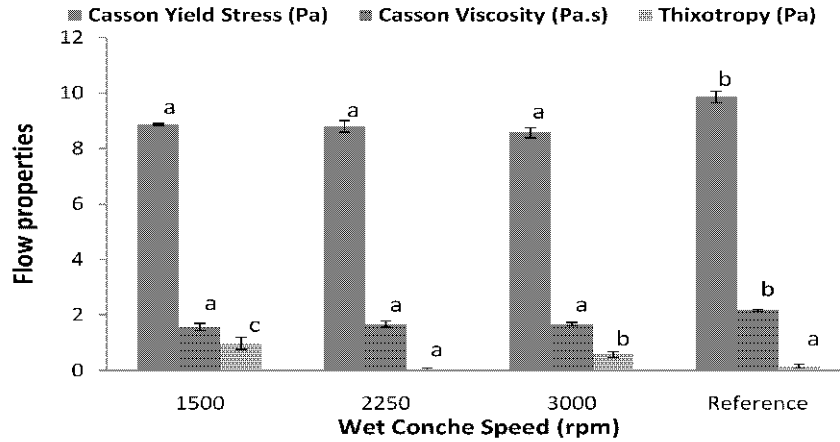
of the particulate system during mixing, with formation of aggregates due to the low rotary speed.

3.4.2.2 Variable rotary speed for wet conching at constant dry conching speeds

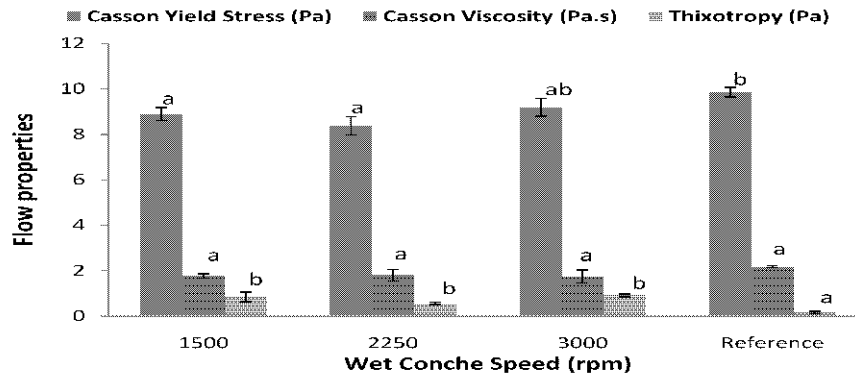
The effect of varying blade rotary speed was investigated for both dry and wet conching processes. This means that the blade rotary speed for dry conching was kept constant while the rotary speed for wet conching was varied. The results are as shown in Figure 3.6 (a-c). Increasing wet conching speed from 1500 to 3000 rpm after dry conching at 3000 rpm did not have any significant ($P>0.05$) effect on the Casson yield value and Casson viscosity (Fig 3.6 a). Thixotropy for the sample wet-conched at 1500 rpm was however significantly higher ($P<0.05$) than that of the samples wet-conched at higher speeds (Fig 3.6 a). Increasing wet conching speed from 1500 rpm to 3000 rpm after dry conching at 2250 rpm had no significant effect on all three flow properties (Fig 3.6 b).

Increasing wet conching speed from 1500 rpm to 2250 rpm after dry conching at 1500 rpm resulted in a significant ($P<0.05$) decrease in both the Casson yield stress and thixotropy (Fig 3.6 c). Overall, samples dry conched at 2250 rpm and 1500 rpm followed by wet conching at 3000 rpm and 1500 rpm, respectively, were not significantly ($P>0.05$) different from the reference sample for Casson yield value and Casson viscosity. Chocolate sample dry conched at 3000 rpm followed by wet conching at 2250 rpm recorded the lowest thixotropy of 0.07 Pa but its Casson yield stress and Casson viscosity was significantly ($P<0.05$) lower than the reference sample.

a.



b.



c.

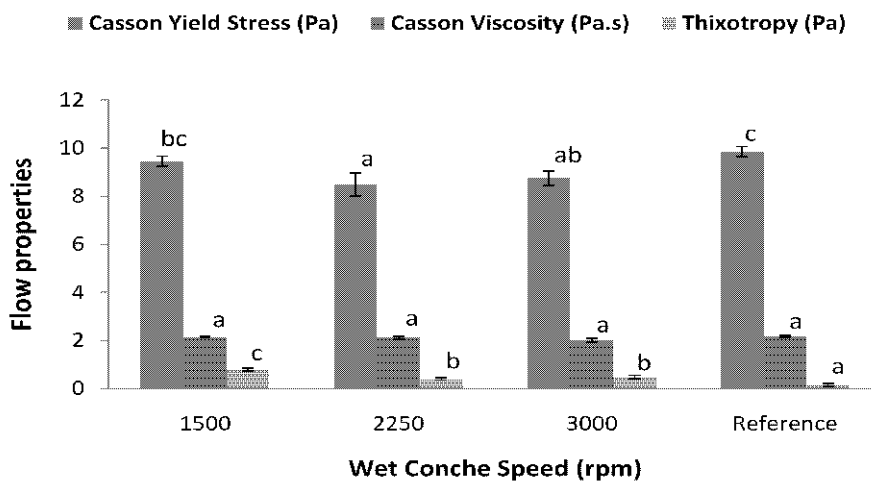


Figure 3.6 Effect of varying blade rotary speed during wet conching at constant dry conching blade speed (a) dry conching rotary speed of 3000 rpm (b) dry conching rotary speed of 2250 rpm (c) dry conching rotary speed of 1500 rpm. [Bars representing a particular flow property, with different letters are significantly ($P \leq 0.05$) different]

3.5 Conclusions

The Stephan mixer was optimized for use as a conche during small scale chocolate production and proved to be a good alternative to the Elk'Oline conche when considering chocolate flow properties. Optimum settings of 65 °C for 10 min at 1500 rpm for dry conching followed by 50 °C for 15 min at 1500 rpm for wet conching resulted in similar flow properties (Casson yield stress and viscosity) as the reference sample although the thixotropy was significantly higher ($P \leq 0.05$) than the reference. Dry conching time showed a significant ($P \leq 0.05$) effect on all chocolate flow properties. The combined influences of time, temperature and blade rotary speed of the Stephan mixer can be manipulated to control flow properties of chocolate when employing the Stephan mixer as a conche. The Stephan mixer is thus very convenient to use, with a great reduction in raw materials use and conching time, and can therefore be used for small scale chocolate processing where only small quantities of molten chocolate are required. The results presented in this chapter cannot be generalized to the whole aspects of the conching process since conching does not only influence chocolate flow properties but is also important for the final texture and flavour of chocolate. The optimization was towards a more quick conching process and not sensory characteristics. Further optimizations regarding moisture balance and flavour development will help improve quality characteristics of products conched with the Stephan mixer.

Chapter 4: Optimization of inulin and polydextrose mixtures as sucrose replacers during sugar-free chocolate manufacture – Rheological, microstructure and physical quality characteristics

This chapter has been published in *Journal of Food Engineering* 126 (2014) 35-42 (Aidoo et al., 2014)

4.1 Introduction

The applicability and suitability of inulin and polydextrose as bulking agents in sugar-free chocolate manufacture have been reviewed (Aidoo et al., 2013). Polydextrose and inulin are considered as fibers that do not only increase the bulk constituent of food and its rapid movement through the gastrointestinal system, but also helps in preventing constipation and possible colon and rectal cancer (Thompson, 2004). Polydextrose, a randomly linked polymer of glucose has similar technological properties as sucrose except for sweetness. Inulin is a polymer of various lengths comprising of fructose molecules linked together and ending with a glucose molecule (Burdock & Flamm, 1999; Afoakwa et al., 2007a; Beckett, 2009).

Polydextrose, as a commercial available preparation, is produced by the condensation of a melt which consists of approximately 89% D-glucose, 10% sorbitol and 1% citric acid on a weight basis (Colliopoulos et al., 1986). Polydextrose has been successfully incorporated into a wide range of foods including baked goods, beverages, confectionery and frozen desserts and is known to provide the bulk and appropriate textural and mouthfeel qualities which are usually associated with sugar and fat while lacking the sweet taste and caloric value connected with those conventional food ingredients (Lauridsen, 2004). Section 2.3.3.1 gives detailed information about polydextrose.

Inulin is a mixture of oligo- and polysaccharides, which are composed of fructose units connected by β - (2-1) links. The extensive use of inulin in the food industry is based on its nutritional and technological properties. Inulin is of interest for the development of healthy products because it simultaneously responds to a variety of consumer demands: it is fibre-enriched, prebiotic, low fat, and low sugar. As a dietary fibre, inulin passes through the digestive tract largely undigested. In the colon it acts as a prebiotic because it is selectively fermented by the beneficial flora, stimulates their growth, and reinforces its action against putrefactive microorganisms (Roberfroid et al., 1998). Detailed information about inulin can be found in section 2.3.3.2 .

Inulin and polydextrose have often formed the basis for most researches for use as bulking agents in the production of sugar-free chocolates (Farzanmehr & Abbasi, 2009; Shah et al., 2010; Pallazo et al., 2011) as reviewed by Aidoo et al. (2013) in section 2.3.5.3. Findings from

these previous researches show that substituting sucrose with these polymers as bulking agents could lead to production of low-calorie chocolate but the functionality of inulin and polydextrose in dark chocolate manufacture and their influences on dark chocolate flow properties have not been fully elucidated. This work was therefore aimed at investigating the optimum conditions as well as influences of inulin and polydextrose mixtures as sucrose replacers on the rheological properties, microstructure and physical qualities during sugar-free dark chocolate manufacture.

4.2 Research strategy

Inulin and polydextrose have in recent times been used as basic ingredients in the manufacture of many sugar-free products. However, the applicability and suitability of inulin and polydextrose mixtures as sucrose replacers during sugar-free chocolate manufacture is yet to be fully understood. This work investigated optimum conditions as well as influences of inulin and polydextrose mixtures as bulk ingredients on the sugar-free dark chocolate microstructure and physical quality characteristics.

4.3 Materials and methods

4.3.1 Raw materials

Cocoa liquor and cocoa butter of Ghanaian origin were obtained from Cargill (Mouscron, Belgium). Sucrose (pre-broken) was supplied by Barry Callebaut (Wieze, Belgium). Soy lecithin (containing 62% acetone-insoluble matter) was supplied by Soya International (Roslyn, USA). Polydextrose (Litesse Two) was supplied by Danisco (Dordrecht, Holland). Inulin (Orafti HP) was supplied by BENEEO Orafti (Tienen, Belgium).

4.3.2 Experimental design and sample preparation

Chocolate samples were prepared at UGent Cacaolab. The chocolates were formulated from cocoa liquor, cocoa butter, lecithin, and bulking agents (inulin, polydextrose) (Table 4.1) at a total fat content of 33% (w/w). Table 4.2 presents the experimental design for 12 formulations which underwent quality analysis. The ingredients were weighed and mixed in

a Vema mixer (Vema BM 30/20, Vemaconstruct, NV Machinery Verhoest, Izegem, Belgium) at a temperature of 45 °C at 3 rpm rotational speed for 20 min. The mixed ingredients were refined using a 3-roll refiner (Exakt SOS Apperatebau GmbH & Co. KG, Norderstedt, Germany) to 28-30 µm particle sizes. Seven hundred (700) grams of refined chocolate was placed in a Stephan mixer (STEPHAN Food Service Equipment GmbH, Hameln, Deutschland) and dry conched at 65 °C for 10 min. Lecithin and cocoa butter were added after dry conching and this was followed by wet conching at 50 °C for 15 min. The resulting molten chocolate obtained was kept in sealed plastic containers at ambient temperature (20-22 °C) for further analysis. A reference chocolate sample was prepared with sucrose instead of inulin and polydextrose mixtures as bulking agents.

4.3.3 Tempering procedure

Samples for hardness measurements were incubated at 52 °C for 4 h for melting prior to tempering. The molten chocolates were hand-tempered using a fishtail spatula (Chocolate World, Antwerp, Belgium) on a marble table. Two thirds (2/3) of molten chocolate at 45 °C was poured onto the marble plate and manipulated skilfully with spatula until a visual increase of chocolate viscosity was obtained. It was then mixed with the remaining molten chocolate (1/3) to a temperature of about 31 °C. Precrystallisation was measured using Aasted Makrovert Chocometer (Aasted-Makrovert Aps, Farum, Denmark) and chocolate was considered well tempered if a Temper index between 4 and 6 was recorded. The tempered chocolate was then moulded using plastic moulds of dimension 102 mm length, 23 mm breadth, and 8 mm height, and allowed to cool in a refrigerator (11 °C) for 1 h before de-moulding. The moulded finished chocolates were packed onto plastic trays and conditioned at ambient temperature (20 ± 2 °C) for 2 weeks before analyzed.

Table 4.1 Ingredients used in dark chocolate formulations

Material	Composition (%)
Cocoa Mass	40
Cocoa butter	11.6
Bulking agent*	48
Lecithin	0.4

*Constitutes mixtures of inulin and polydextrose or sucrose (Table 4.2)

Table 4.2 Experimental design for bulking agent components in dark chocolate formulation

Formulation	Inulin (%)	Polydextrose (%)	Sucrose (%)
1	25	75	0
2	50	50	0
3	75	25	0
4	100	0	0
5	0	100	0
6	50	50	0
7	100	0	0
8	25	75	0
9	75	25	0
10	0	100	0
11	0	0	100
12	0	0	100

4.4 Analytical methods

4.4.1 Rheological properties

The method is as outlined in Section 3.3.4.

4.4.2 Moisture

The Karl-Fisher titration method (ICA 26, 1988) was used to determine moisture content of samples. Chocolate samples in sealed plastic containers were heated for at least an hour in an oven at 60 °C to melt before measurements were conducted. Mean values from 3 replicate measurements and standard deviations were calculated.

4.4.3 Particle size distribution (PSD)

The particle sizes were measured using a Malvern MasterSizer equipped with a 300 RF lens to measure particles in a range of 0.05–900 µm. Approximately 0.5 g of molten chocolate was mixed with 10 ml of isopropanol and placed in an oven at 60 °C for approximately one hour shaking vigorously to aid dissolution. Drops of the dissolved chocolate was dispersed in isopropanol at ambient temperature (20 ± 2 °C) until an obscuration between 10 – 30 % was obtained. The sample was continuously shaken during measurement to ensure particles were independently dispersed. Size distribution was quantified as the relative volume of particles

in size bands presented as size distribution curves. Mean values of the largest particle size (D_{90}) from 5 replicate measurements and standard deviations were calculated.

4.4.4 Hardness

The hardness of chocolate bars was measured with TA.XTplus texture analyser (Lloyd Instruments, West Sussex, UK) with a load cell of 500 N and needle geometry. Hardness was reported as the maximum penetrating force (N) required for the needle to penetrate through the chocolate sample (102 x 23mm, depth 8mm) at 20 °C, over a distance of 5 mm at a constant speed of 2mm/s. Mean values from 8 replicate measurements and standard deviations were recorded.

4.4.5 Colour

Colour of chocolate bars was measured with a colorimeter, Minolta Model CM-2500D Spectrophotometer (Konica Minolta Sensing. Inc., Osaka, Japan) calibrated with white reference standard. The SCE-mode (Specular light excluded) was used with the colour expressed in terms of the CIELAB system L^* , a^* and b^* : L^* , luminance ranging from 0 (black) to 100 (white); and a^* (green to red) and b^* (blue to yellow). Mean values from 5 replicate measurements and standard deviations were calculated.

4.4.6 Microscopy

Microstructures were observed using a high resolution polarized light microscope (Leitz Diaplan, Pleitz Wetzlar, Germany) with Olympus colour view camera (Olympus, Aartselaar, Belgium) and a temperature-controlled stage Linkam PE94 (Linkam Scientific Instruments, Surrey, UK). Chocolate samples were first melted in an oven at 52 °C for 4 hours to destroy crystal memory. A drop of molten chocolate was brought onto a glass slide using a Pasteur pipette and carefully covered with a cover slip to prevent the occurrence of air bubbles. Samples were observed immediately at a x10 magnification and micrographs (black and white images) were captured using a digital camera (Olympus, Aartselaar, Belgium) and analysed using the Cell[^]D Imaging software.

4.5 Data analysis

Data was analyzed using Statgraphics Centurion XV (Graphics Software System, STCC, Inc., Rockville, USA). Chocolate properties (rheology, hardness, colour, D_{90} , moisture) were subjected to variance analysis (ANOVA) and multiple range tests to determine effect of the bulking agent concentrations on the studied parameters. Tukey's honestly significant multiple comparisons (95% significance level) determined differences between factor levels.

4.6 Results and discussion

Table 4.2 presents the experimental design for 12 formulations which underwent quality analysis. Experiments 11 and 12 represent the control formulation made with only sucrose. Pearson's correlation coefficients determined by analysis of variance (ANOVA) are presented in Table 4.3. Pearson's correlation coefficients range between -1 and +1 and measure the strength of the linear relationship between two variables, where 1 is total positive correlation, 0 is no correlation, and -1 is total negative correlation.

4.6.1 Rheological properties

4.6.1.1 Casson plastic viscosity

The rheological properties of the molten chocolate samples were characterized using the Casson model. Chocolate flow curve for the reference dark chocolate showing the measurement of shear stress as a function of increasing shear rate from 2 s^{-1} to 50 s^{-1} (ramp up), holding at 50 s^{-1} for 60 s, then decreasing from 50 s^{-1} to 2 s^{-1} (ramp down) is shown in Fig. 4.1.

Increasing inulin concentration with simultaneous reduction in polydextrose concentration resulted in increases in the Casson plastic viscosity (Fig. 4.2). The highest viscosity was achieved by substituting sucrose completely with inulin. In contrast, chocolate formulations with high levels of polydextrose (75 and 100%) presented the lowest Casson plastic viscosity (Fig. 4.2). Casson plastic viscosity relates to pumping characteristics, filling of rough surfaces, coating properties and sensory character of body (Seguine, 1988). Casson viscosity reference values between 2.1 and 3.9 Pa.s have been reported by Aeschlimann & Beckett (2000) for dark chocolates. All chocolate formulations with the exception of samples containing

moderate (50%) to high levels (75% and 100%) of inulin were within the range reported viscosity (Fig. 4.2). This means that only chocolate formulations with inulin concentrations ($\leq 25\%$) according to the experimental design can be employed for enrobing or coating.

Table 4.3 Pearson's correlation matrix for dark chocolate properties

	Casson viscosity	Casson Yield	Colour	D ₉₀	Hardness	Moisture
Casson viscosity		-0.8789*	0.0533	0.8895*	0.4779	-0.0149
Casson Yield	-		-0.3271	-0.8085*	-0.4488	0.0899
Colour	-	-		0.2945	-0.3546	-0.0117
D ₉₀	-	-	-		0.2787	0.1409
Hardness	-	-	-	-		-0.1841
Moisture	-	-	-	-	-	

*Significant at $P \leq 0.05$

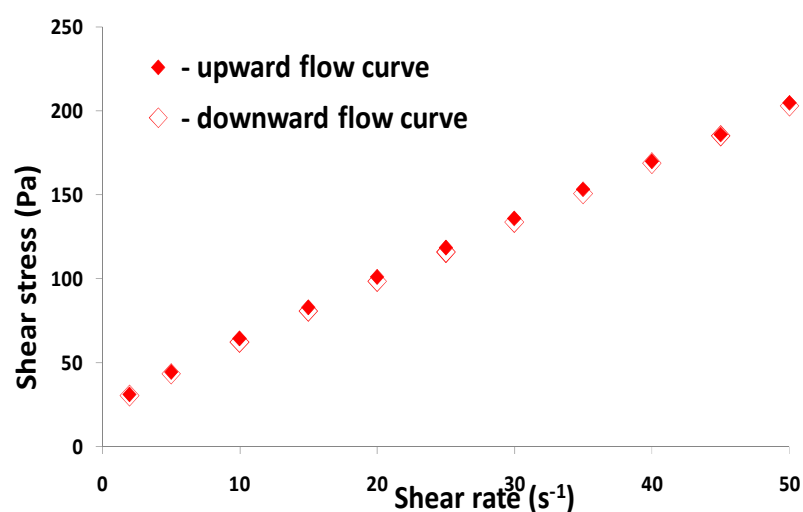


Figure 4.1 Flow curve for the reference dark chocolate showing shear stress as a function of shear rate

Shah et al. (2010) reported a higher plastic viscosity for chocolates with inulin HP (average DP ≥ 23 , long chain inulin) compared to their control sample made with sucrose. This confirms our findings since inulin HP was used in our study. ANOVA showed that total sucrose replacement with polydextrose did not have any significant ($P < 0.05$) effect on the Casson viscosity (Fig. 4.2). The Casson viscosity for chocolate formulation with 100% inulin

was however significantly ($P < 0.05$) higher than all other sugar substituted chocolates as well as the reference sample (Fig. 4.2). The results also showed that a mixture of 75% polydextrose and 25% inulin can replace sucrose in dark chocolate without any significant ($P < 0.05$) change in the Casson viscosity (Fig. 4.2).

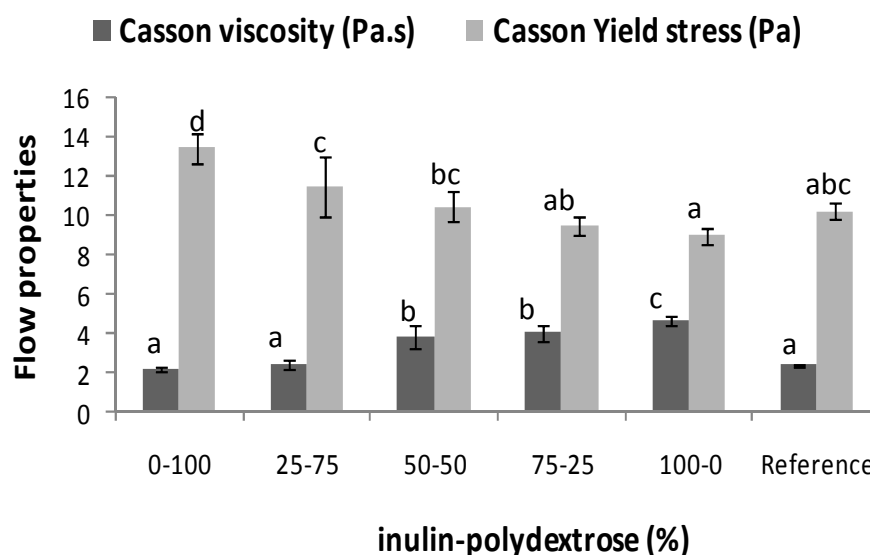


Figure 4.2 Effect of sugar substitutes concentration on flow properties of chocolate. [samples with different letters are significantly ($P \leq 0.05$) different, for each flow property]

4.6.1.2 Casson yield stress

Casson yield stress is the force required to initiate flow of molten chocolate. It represents the low shear-rate properties of chocolate and is affected by particle–particle interaction, the amount and specific surface area of the particles, emulsifiers, and moisture (Servais et al., 2004; Afoakwa et al., 2007; Aidoo et al., 2013). The Casson yield numbers for all formulations were within the range reported for dark chocolate, i.e. 4–32 Pa (Aeschlimann & Beckett, 2000). A general trend emerged where decreasing polydextrose concentration with a simultaneous increase in inulin concentration led to a decrease in the Casson yield stress (Fig. 4.2). The lowest value of 8.74 Pa and highest value of 13.32 Pa was recorded for samples containing 100% inulin and 100% polydextrose, respectively. ANOVA showed that the Casson yield stress for the reference sample was not significantly ($P > 0.05$) different from samples containing mixtures of polydextrose and inulin (Fig. 4.2). Thus, a mixture of 75%

polydextrose and 25% inulin can replace sucrose in dark chocolate without significant ($P < 0.05$) change in the Casson yield stress as observed also for the Casson viscosity. Sucrose replacement with 100% polydextrose however recorded significantly ($P < 0.05$) higher casson yield stress than all the other sugar substituted chocolates. A significant ($P < 0.05$) correlation ($r = -0.88$, $p = 0.0008$) was noted between Casson viscosity and Casson yield stress (Table 4.3) indicating a strong linear relationship between both variables.

Rheologically, chocolate properties are mainly influenced by particle size distribution and ingredients composition. Viscosity of suspensions can be greatly modified by changing particle size distribution (PSD) while maintaining the same solid content (Afoakwa et al., 2008; Aidoo et al., 2013). Variations in particle size distribution (PSD) were observed for the chocolate samples using the largest particle size (D_{90}) values (90% of all particles have smaller sizes than given value) which correlates fairly on sensory character (Beckett, 2000). D_{90} values for formulations containing high levels of inulin ($\geq 50\%$) were significantly ($P < 0.05$) higher compared to the reference sample (Fig 4.3).

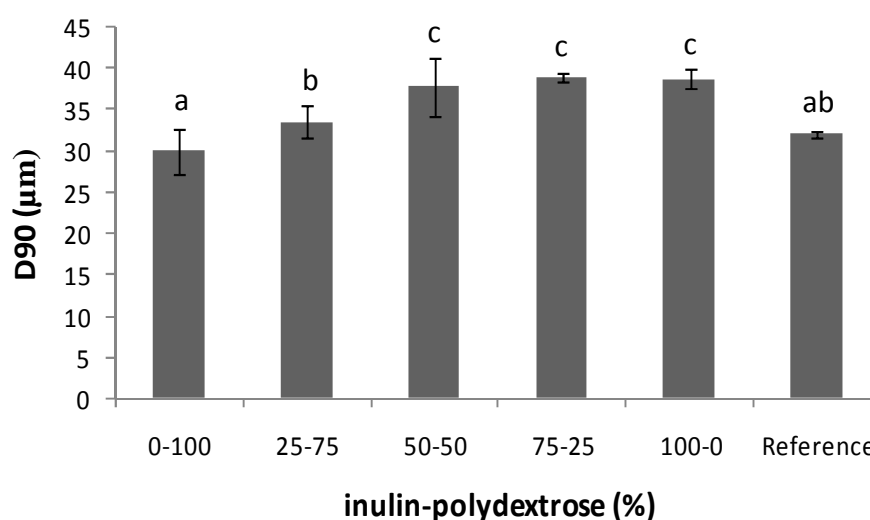


Figure 4.3 Effect of sugar substitutes concentration on particle size (D_{90}) of chocolate
[Samples with different letters are significantly ($P \leq 0.05$) different]

Figure 4.4 shows volume histograms of the different chocolate formulations. Formulations with 100% polydextrose showed a narrow unimodal distribution compared to the reference sample which presented a more broader bimodal distribution (Figure 4.4). PSD of unimodal distributions are known to have greater influence on yield stress due to their smaller particle

sizes resulting in increased surface areas of particles and inter-particle interactions. This explains the high yield stress recorded for formulations containing 100% polydextrose. All other formulations containing inulin showed bimodal or trimodal distributions (Figure 4.4).

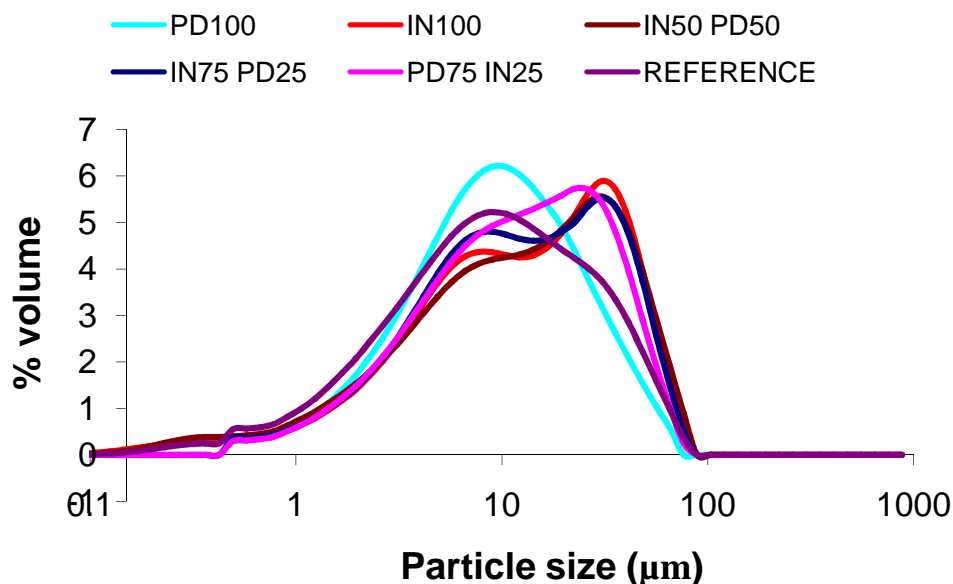


Figure 4.4 Effect of sugar substitutes concentration on Particle Size Distribution of dark chocolate. IN = inulin; PD = polydextrose

Careful observation of the volume histograms showed a gradual decrease in bimodality of samples with increase in polydextrose concentration. This further explains the decrease in Casson yield stress with decrease in polydextrose concentration. Chevalley (1994) showed that as the particle size distribution becomes broader in the direction of smaller particles, increase in Casson yield stress is observed. This is due to an increase in the internal surface area, and therefore either the number of bonds or frictional contact between the particles is higher.

Optimization of PSD leads to viscosity reduction without significant modification to the overall chocolate formulation, which can be economically beneficial to the manufacturer. Broader PSD increases polydispersity and higher fractions of coarser particles than finer particles lowers viscosity by decreasing mean particle surface area, which consequently reduces the total amount of fat needed for particle coating and thus increases fat content needed for good flow of suspension (Mongia and Ziegler, 2000). Sokmen and Gunes (2006)

however noted that although the particle size of isomalt in their study was higher than the other sugar alcohols and sucrose, it did not show a lower viscosity as was expected. A similar trend was observed in our study where inulin samples with the largest particle sizes recorded the highest viscosity. Variations in the particle size distribution as explained by composition of the sugar replacers present could also be attributed to difficulties in the conching process. Significant correlation coefficients were noted between D_{90} and Casson yield stress ($r = -0.81$, $p = 0.005$) and D_{90} and Casson viscosity ($r = 0.89$, $p = 0.0006$) (Table 4.3). This suggests that both Casson yield stress and Casson viscosity could be related to distribution of particle sizes and strength of the aggregated particle-to-particle network system of the chocolate mass during sugar-free chocolate manufacture (Servais et al., 2004; Beckett, 2000).

4.6.2 Microscopy

To further explain the above rheological trends, light microscopy was used to characterize the network structure of the chocolate formulations. Micrographs (Figure 4.5A-4.5F) showed clear variations in microstructure for the different chocolate formulations. Chocolate formulations containing 100% polydextrose (Figure 4.5B) showed a dense structure of crystal network with minimal inter-particle spaces (dark spots). However, formulations containing 100% inulin (Figure 4.5 (C)) revealed large crystals with more void spaces (dark spots) between the crystals indicating limited particle - particle interaction strength. The high solids packing intensity of 100% polydextrose chocolate formulations could have resulted in higher energy needed to initiate flow, hence, higher Casson yield stress values as compared to chocolate formulations containing 100% inulin. The micrographs also showed larger crystals for samples containing 50% inulin : 50% polydextrose (Figure 4.5 (F)) as compared to samples containing 75% inulin : 25% polydextrose (Figure 4.5 (E)). Formulations containing 75% inulin : 25% polydextrose also showed a high solids packing intensity (Figure 4.5 (E)) than 50% inulin : 50% polydextrose (Figure 4.5 (F)) formulations. This could explain the increase in Casson viscosity with increase in inulin concentration since the dense packing of samples containing high levels of inulin may limit flow of molten chocolate. Inulin can thus not be used alone in sugar-free chocolate manufacture due to its significant influence on

viscosity and will need to be combined with other bulking agents such as polydextrose to reduce product viscosity.

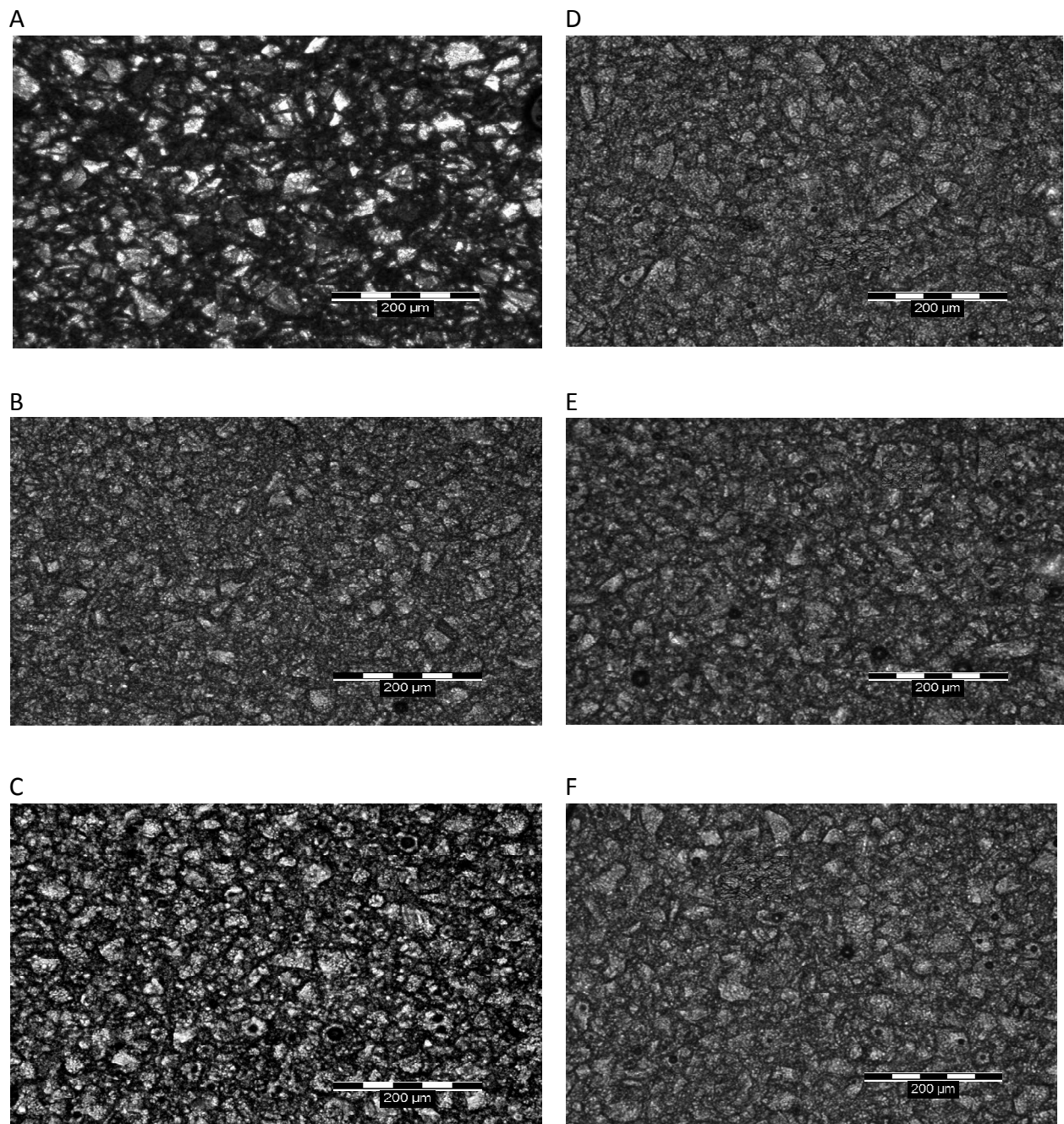


Figure 4.5 Micrographs of molten chocolates obtained with Cell^{AD} Imaging software A. Sucrose B. Polydextrose C. Inulin D. 25% inulin : 75% polydextrose E. 75% inulin : 25% polydextrose F. 50% inulin : 50% polydextrose

4.6.3 Colour

Colour is one of the key attributes for consumer acceptance. Many visual attributes can be used to describe the appearance of chocolate which includes gloss, shape, surface smoothness or roughness, haze, translucency and colour (Briones et al., 2006). Colour changes in chocolate are often due to the difference in composition and processing parameters during production. Chocolate colour varied significantly among the different formulations (Fig 4.6). Lower values for L* (lightness) indicate a darker appearance. Generally, replacing sucrose with inulin and polydextrose resulted in darker chocolates compared to the reference, regardless of the levels of the sugar substitutes used. This is in agreement with the study of Shah et al. (2010) who reported darker colours for chocolates containing inulin (HP, HPX and GR) with different degrees of polymerization and polydextrose as bulking agents.

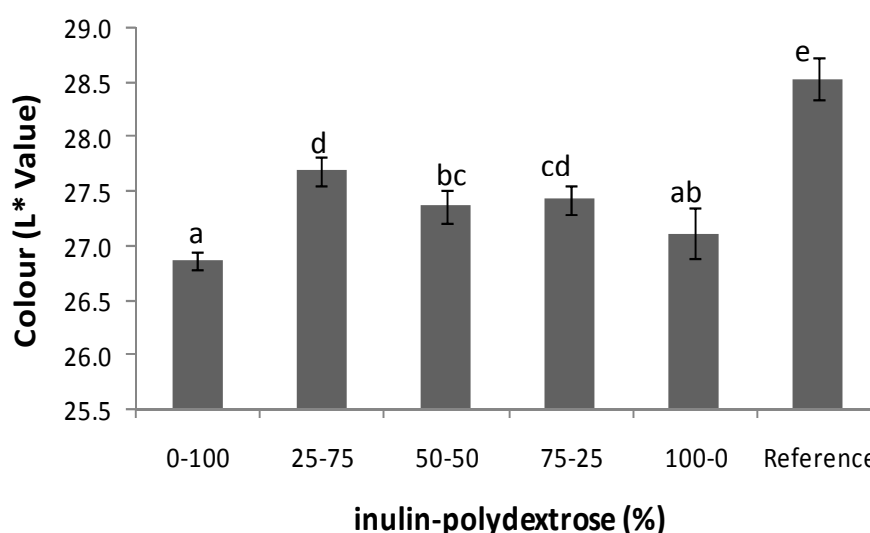


Figure 4.6 Effect of sugar substitutes concentration on colour of chocolate [Samples with different letters are significantly ($P \leq 0.05$) different]

Chocolate samples containing high levels (100%) of the sugar substitutes presented the darkest chocolates. This is a well reported phenomenon, as the addition of polysaccharides accelerates caramelization and Maillard reaction and therefore speeds up the formation of chocolate colour. ANOVA showed that all sugar substituted samples were significantly ($P < 0.05$) darker in colour than the reference sample. The colour of formulation containing

100% polydextrose was not significantly ($P>0.05$) different from colour of formulation containing 100% inulin.

4.6.4 Hardness

Chocolate hardness varied significantly among the individual samples. High levels of the sugar substitutes led to a hardening effect on the chocolates (Fig 4.7). Formulations containing equal concentrations of both sugar substitutes recorded the least hardness (3.81 N) (Fig 4.7). Overall, formulations containing 100% inulin was the hardest with an average of 5.32 N compared to the reference (approx. 3.79 N). Farzanmehr and Abbasi (2009) reported chocolate formulation with 50% inulin: 25% polydextrose: 25% maltodextrin as the hardest indicating the dominant effect of inulin in the formulation as recorded in our study.

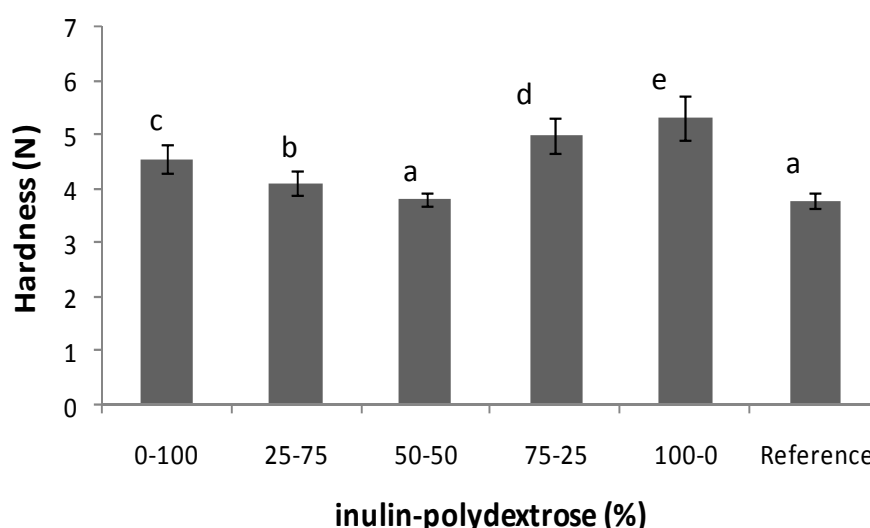


Figure 4.7 Effect of sugar substitutes concentration on hardness of chocolate [Samples with different letters are significantly ($P\leq0.05$) different]

Replacement of sucrose with inulin and polydextrose as bulking agents in the study of Shah et al. (2010) however had no substantial effect on chocolate hardness except for chocolates made with inulin HPX which was not used in our study. Afoakwa et al. (2007) noted that several factors including recipe, manufacturing techniques, temper, polymorphism (stability of fat crystals) and cooling temperature controls influence final texture (hardness) of solid

tempered chocolate. Keogh et al. (2002) also concluded that hardness is a useful indicator of good tempering, or degree to which a stable fat crystal network has been formed. The differences observed in the chocolate hardness can thus be attributed to the tempering process as well as influences of the bulking agents and their particle size distributions. ANOVA showed that all samples were significantly ($P < 0.05$) different from each other for hardness. Sucrose replacement with equal concentrations of polydextrose and inulin however had no significant ($P > 0.05$) effect on chocolate hardness.

4.6.5 Moisture

The moisture content in chocolate is an important factor as it is closely related to rheological and textural properties. Moisture content of the formulations ranged between 0.60 and 0.83%, which is within the acceptable limit ($< 1\%$), with the reference sample recording an average of 0.46% (Fig 4.8). Farzanmehr and Abbasi (2009) also reported higher moisture content for chocolate formulations containing high levels of maltodextrin, inulin and polydextrose.

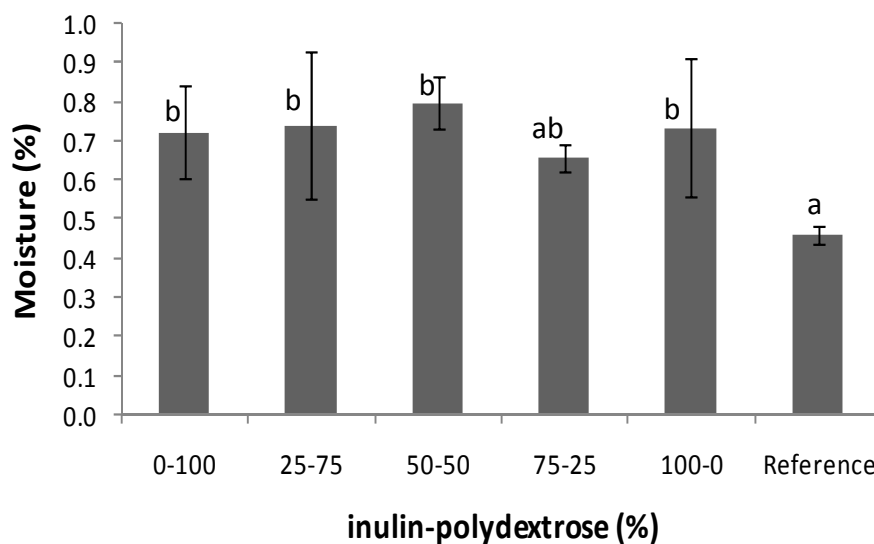


Figure 4.8 Effect of sugar substitutes concentration on moisture levels of chocolate
[Samples with different letters are significantly ($P \leq 0.05$) different]

Molten chocolate typically has moisture contents of 0.5-1.5%, mainly in the cocoa solids, that does not affect chocolate flow. High moisture levels aggregate sugar particles to form gritty lumps and moisture at sugar particle surfaces increases friction between the particles, resulting in increase in apparent viscosity. It is therefore important that as much 'free' water is removed as possible. Our results however showed no significant correlation between chocolate moisture levels and chocolate flow properties (Casson yield stress and viscosity) (Table 4.3). ANOVA showed that the moisture contents of all sugar substituted chocolates were significantly ($P < 0.05$) higher than the reference sample (Fig 4.8) with the exception of the formulation containing 75% inulin and 25% polydextrose which was not significantly different from the reference sample.

4.7 Conclusions

Substitution of sucrose with inulin and polydextrose in sugar-free dark chocolates has varied influences on the rheological properties and physical quality characteristics. The effect is dependent not only on the type of sugar substitute but also on the concentrations present. Increasing inulin concentration resulted in increase in Casson viscosity and reduction in Casson yield stress. Different combinations of inulin and polydextrose can thus be used to improve the rheological properties of sugar-free dark chocolate. All sugar substituted chocolates were darker in colour with high moisture contents compared to the reference sample. A mixture of 75% polydextrose and 25% inulin was selected as the optimum blend for sugar-free dark chocolate based on all studied parameters. Formulations with this blend recorded no significant differences in Casson yield stress, Casson viscosity and D_{90} values compared to the reference sample. Although the resulting chocolate was slightly harder than the reference sample, it presented a much less darker colour compared to the other sugar substituted chocolates. The D_{90} value showed significant correlations with Casson viscosity and Casson yield stress. Thus, further research is needed to understand the effect of particle size distribution on the flow and mechanical properties of sugar-free dark chocolate with inulin and polydextrose mixtures as bulking agents.

**Chapter 5: Impact of particle size distribution and fat content
on rheological, mechanical and melting properties of sugar-
free dark chocolates processed using inulin and polydextrose
mixtures as bulking agents**

This chapter has been submitted to Journal of Food Engineering and is currently under review

5.1 Introduction

A widely appreciated example of a solid suspension is chocolate, which is a complex multiphase system based on solid particles (sugar, cocoa and milk solids) dispersed in a continuous phase, mainly cocoa butter (Afoakwa et al., 2007a; Beckett, 2009). The composition of sucrose in chocolate is about 30-60% and this confers multiple sensorial properties on chocolate including sweetness and mouthfeel (texture) (Jeffery, 1999; Afoakwa et al., 2007a,b; Afoakwa, 2010). Chocolate normally contains 30% to 40% wt fat which plays a major role in the molten chocolate viscosity. Taking out a fraction of the suspending medium therefore causes significant changes in the chocolate viscosity leading to difficulties in processing (Do et al., 2007).

Sugar-free chocolates have become popular among consumers and manufacturers due to the reduced caloric values, non cariogenicity and suitability for diabetics. The applicability and suitability of inulin and polydextrose as bulking agents in sugar-free chocolate manufacture have been reviewed (Aidoo et al., 2013). Both polymers have been successfully incorporated into a wide range of foods including baked goods, beverages and confectionery providing the bulk and appropriate textural and mouthfeel qualities usually associated with sugar and fat while lacking the sweet taste and caloric value connected with those conventional food ingredients (Lauridsen, 2004).

Quality in finished chocolate is highly dependent on inherent size distribution of solid particles from sugar, milk and cocoa, composition of fat phase and emulsifiers (Ziegler, 1992; Beckett, 2003; Afoakwa et al., 2007b). Particle size distribution (PSD), fat content, emulsifiers and processing parameters such as conching influence chocolate properties (Tscheuschner, 1979; Beckett, 1999; Schantz, 2005). Good dark chocolate requires a maximum of 35 μm particle size (Awua, 2002). At solids > 61% by volume and particle size > 35 μm , the quality becomes unacceptable due to high viscosity and poor texture (Beckett, 1999). The largest particles (D_{90}) are important for mouthfeel notably grittiness, but smaller particles influence flow properties (Beckett, 2003; Beckett, 2000; Mongia, 2000; Ziegler et al., 2001). Limit values are determined by targets for character and product composition.

5.2 Research strategy

Several examples of PSD optimization to improve process efficiency and/or yield in food manufacture have been published (Missaire et al., 1990; Aguilar et al., 1991; Villagran et al., 1996). Several researches on the manipulation of particle size distribution to optimize flow properties of conventional chocolate have also been published (Servais et al., 2002; Do et al., 2007; Afoakwa et al., 2007b; Afoakwa et al., 2008). There is however limited study of PSD effect on sugar-free chocolates and its effect on products quality characteristics. Earlier studies reported changes in flow properties with variations in inulin and polydextrose concentrations in dark chocolate formulations (Aidoo et al., 2014a). The authors attributed this to the packing ability of the solids with micrographs showing clear variations in the microstructural behaviour of inulin and polydextrose in chocolate formulations (Aidoo et al., 2014a). The objective of this study was to investigate the effects of fat content and PS (particle size) on the rheological, mechanical and melting properties of sugar-free chocolates produced with polydextrose/inulin mixtures as bulking agents. With opportunity for improvements in quality of reduced calorie chocolates through new product development, understanding the factors influencing the rheological, mechanical and melting properties of sugar-free chocolates would have significant industrial applications.

5.3 Materials and methods

5.3.1 Raw materials

Cocoa liquor and cocoa butter of Ghanaian origin were obtained from Cargill (Mouscron, Belgium). Sucrose (pre-broken) was supplied by Barry Callebaut (Wieze, Belgium). Soy lecithin (containing 62% acetone-insoluble matter) was supplied by Soya International (Roslyn, USA). Polydextrose (Litesse Two) was supplied by Danisco (Dordrecht, Holland). Inulin (Orafti HP) was supplied by BENEEO Orafti (Tienen, Belgium). Stevia rebaudioside A (Eureba Reb A97) was obtained from Bayn (Stockholm, Sweden).

5.3.2 Chocolate production

Chocolate samples were prepared according to the formulations in Table 5.1. The ingredients were mixed in a planetary Vema mixer BM 30/20 (Vemaconstruct,NV machinery

Verhoest, Izegem, Belgium) and refined to pre-determined particle sizes (D_{90} sizes of $20 \pm 1 \mu\text{m}$, $30 \pm 1 \mu\text{m}$ and $50 \pm 1 \mu\text{m}$) with an Exakt 80S 3-roll refiner (Exakt Apparatebau GmbH & Co. KG, Norderstedt, Germany). To attain such particle size ranges, the rolling process had to be optimized. Roller settings of “3 - 1 x 2 - 1”, “3 - 1 x 3 - 1” and “4 - 1 x 4 - 1” were obtained for D_{90} sizes of $20 \pm 1 \mu\text{m}$, $30 \pm 1 \mu\text{m}$ and $50 \pm 1 \mu\text{m}$, respectively. A roller setting of “4 - 1 x 4 - 1” means the sample was first refined using settings of “4 - 1” and then there is a second refining with settings of 4 - 1. Each sample was thus refined twice. The refined chocolates were dry conched in a Buhler Elk’Olino conche (Richard Frisse GmbH Bad Salzungen, Germany) for 6h (clockwise mixing: – 1200 rpm, 60 °C, 120 min; anti-clockwise shearing: 1200 rpm, 70 °C, 120 min) with addition of 1% cocoa butter to aid shearing. Lecithin and the remaining cocoa butter were added prior to wet conching at high speed (clockwise mixing: 2400rpm, 45 °C, 15 min; anti-clockwise shearing: 2400 rpm, 45 °C, 15 min) to obtain optimum mixing and liquefaction. The resulting molten chocolate obtained was kept in sealed plastic containers at ambient temperature (20-22 °C) for further analysis.

5.3.3 Tempering procedure

Samples for hardness measurements were incubated at 52 °C for 4 h for melting prior to tempering. The molten chocolate was tempered using a Selmi One Continuous Chocolate temper machine (Selmi Srl, Santa Vittoria d’Alba (CN), Italy) and precrystallisation was measured using Aasted Makrovert Chocometer (Aasted-Makrovert Aps, Farum, Denmark). Chocolate was considered well tempered if a Temper index between 4 and 6 was recorded. The tempered chocolate was then moulded using a plastic mould of dimension 102 mm length, 23 mm breadth, and 8 mm height, and allowed to cool in a refrigerator (11 °C) for 1 h before de-moulding. The moulded finished chocolates were packed onto plastic trays and conditioned at ambient temperature ($20 \pm 2 \text{ °C}$) for 2 weeks before analyzed.

5.4 Analytical methods

5.4.1 Particle size analysis

The method is as outlined in Section 4.4.3

5.4.2 Rheological properties

The method is as outlined in Section 4.4.1

5.4.3 Hardness

The method is as outlined in Section 4.4.4

5.4.4 Melting properties

The melting properties of chocolate samples were measured using a Differential Scanning Calorimeter (DSC) (TA Instruments, New Castle, USA). Approximately 5 mg of tempered chocolate samples were weighed into Aluminium pans (TA Instruments, New Castle, USA). The hermetically sealed pans were then heated from 15 to 65 °C at 5°C/min in the DSC using an empty aluminum pan (TA Instruments, New Castle, USA) as reference. The Onset temperature (T-onset), Peak temperature (T-peak), peak width at half height (T-width) and enthalpy of melting (ΔH_{melt}) were automatically calculated after integrating the melting peaks using TA Data analysis software (TA Instruments, New Castle, USA).

5.5 Statistical analysis

Data was analyzed using Statgraphics Centurion XV (Graphics Software System, STCC, Inc., Rockville, USA). The rheological, mechanical (hardness) and melting properties (T-onset, T-max, T-width and ΔH_{melt}) were analysed using analysis of variance (ANOVA) and multiple range tests to determine effects of particle size (PS) and fat content and their interactions on the studied parameters. Tukey's honestly significant multiple comparisons (95% significance level) determined differences between factor levels.

5.6 Results and discussion

Table 5.1 presents the formulations used in chocolate manufacture. Table 5.2 presents the data for melting properties of the developed sugar-free dark chocolates and Figure 5.1 shows the relative and cumulative distribution curves for the different particle size ranges. Chocolates refined to particle sizes of 20 μm and 30 μm showed a bimodal distribution. The

largest particle size (50 μm) showed a trimodal distribution (Fig. 5.1A). Data from the PSD showed variations in Sauter mean $D[3,2]$, mean particle $D[4,3]$, D_{10} (10% of all particles finer than this) and D_{50} (50% of all particles finer than this) with increasing particle size. The D_{90} value (90% of all particles have smaller sizes than given value) was used as it correlates fairly on sensory character with micrometer measurements made of the biggest particles (Afoakwa et al., 2007b; Afoakwa, 2010).

Table 5.1 Recipe used in dark chocolate formulation for the different fat content

Ingredient	Fat (% w/w)		
	33	30	27
Cocoa liquor (%)	40	38	36
Cocoa butter (%)	11.6	9.6	7.6
Bulking agent (%) (Inulin/Polydextrose) ^a	47.76	51.76	55.76
Stevia (sweetener) (%)	0.24	0.24	0.24
Soy lecithin (%)	0.4	0.4	0.4

^aRatio of polydextrose : inulin is 75:25 %

5.6.1. Rheological properties

5.6.1.1 Casson viscosity

The Casson viscosity showed a general trend of increasing with decreasing fat levels at all particle sizes (Fig. 5.2). Fat fills spaces or voids between particles in molten chocolate and reduces resistance to flow. Decreasing fat content from 33% to 30% at 20 μm resulted in 44% increase in Casson viscosity where as a further decrease in fat content from 30% to 27% at 20 μm resulted in 51% increase in Casson viscosity. Similar observations were made for particle sizes of 30 μm and 50 μm where increase in Casson viscosity with decrease in fat content from 33% to 30% was of a lower margin than from 30% to 27%. Servais et al. (2002) reported that viscosity can double with solid content increases of a few percent for high solid content suspensions.

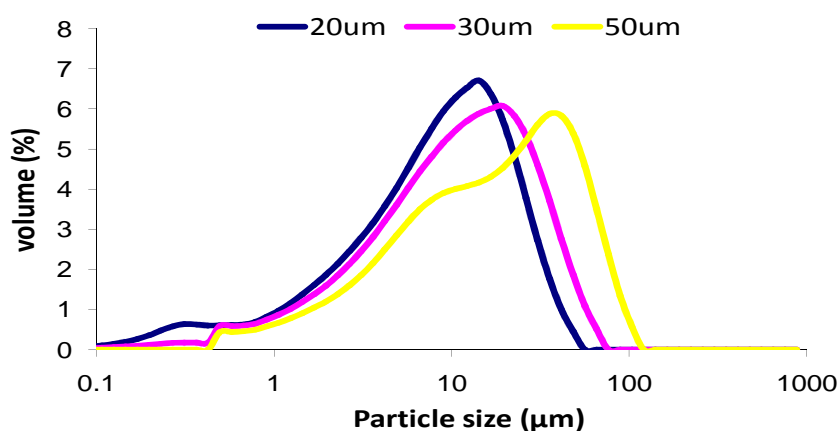
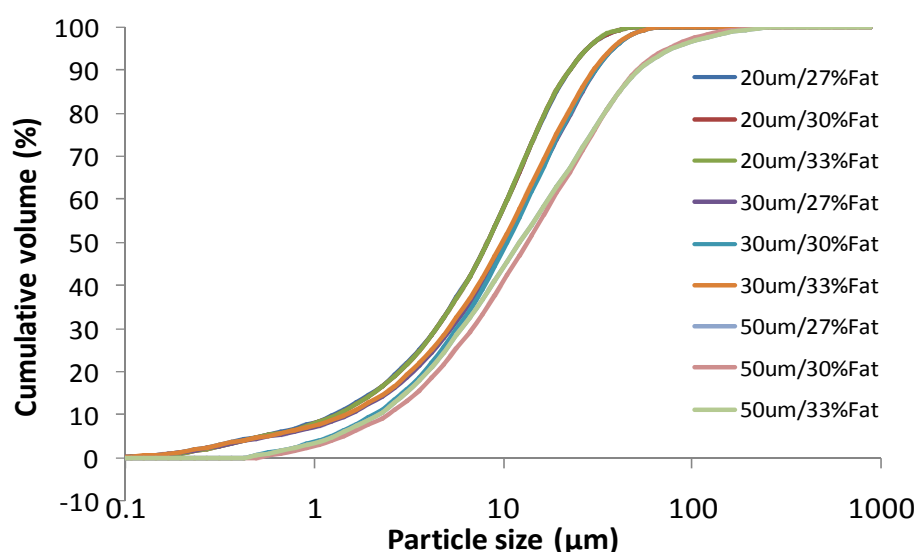
A**B**

Figure 5.1 (A) Volume histograms showing the particle sizes distributions (PSD) at 30% fat (B) Cumulative distribution curves showing the different PSD for all chocolate samples.

Casson plastic viscosity values of 2.1 and 3.9 Pa.s have been reported to be the acceptable minimum and maximum for dark chocolates (Aeschlimann & Beckett, 2000). The results showed that all samples processed at 33% fat content fell within range but the low fat chocolates recorded Casson viscosity values which were out of range (between 4.56 Pa.s and

6.32 Pa.s at 30% fat and between 9.29 Pa.s and 14.77 Pa.s at 27% fat). Such high viscosity means the formulations at 30% and 27% fat cannot be employed for enrobing or coating with requirements for smoother and thinner chocolates.

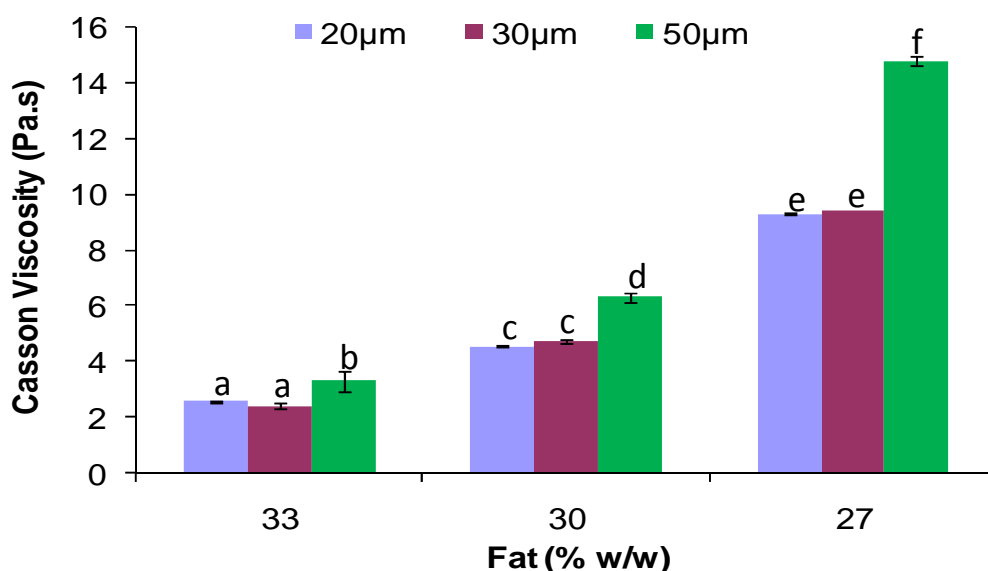


Figure 5.2 Effect of particle size on Casson viscosity of sugar-free chocolates with polydextrose/inulin mixtures at different fat contents [samples with different letters are significantly ($P \leq 0.05$) different]

Particle size effect on Casson viscosity was more pronounced at lower fat contents than at higher fat contents. The Casson viscosity increased from 2.56 Pa.s at 20 µm to 3.30 Pa.s at 50 µm for 33% fat level, representing a percent increase of 22.42% as compared to an increase from 9.29 Pa.s at 20 µm to 14.77 Pa.s at 50 µm for 27% fat level, representing a 37.10% increase in Casson viscosity. Broader particle size distribution increases polydispersity and higher fractions of coarser particles than finer particles lowers viscosity by decreasing mean particle surface area, which consequently reduces the total amount of fat needed for particle coating leaving a larger fraction to aid the flow of suspension (Mongia and Ziegler, 2000). Sokmen and Gunes (2006) however noted that although the particle size of isomalt in their study was larger than the other sugar alcohols and sucrose, it did not show a lower viscosity as was expected. Earlier studies reported a similar trend where chocolate samples

made with inulin recorded the highest viscosity even though it had the largest particle size (Aidoo et al., 2014a).

As earlier mentioned, samples with particle sizes of 20 μm and 30 μm showed a bimodal distribution (fig 5.1), indicating a distribution of two particle sizes in suspension. Such broad particle size distributions according to Do et al. (2007), could lower viscosity because there is the probability of smaller particles filling the gaps between the larger ones, reducing the amount of suspending medium needed to fill the voids. Based on mathematical considerations, Farris (1968) showed that there is an optimum blend of fine and coarse particles for bimodal distributions that maximizes the particle volume fraction and therefore minimizes the viscosity.

Another important parameter to consider in discussing packing volumes and viscosities is the particle shape. Microscopic images of raw ingredients of inulin and polydextrose are shown in Figure 5.3. Polydextrose is observed as non-spherical and angular with variable shapes (Figure 5.3A) whereas inulin is more rounded at the edges (spherical) with evenly distributed sizes and appear mostly in clusters (Figure 5.3B).

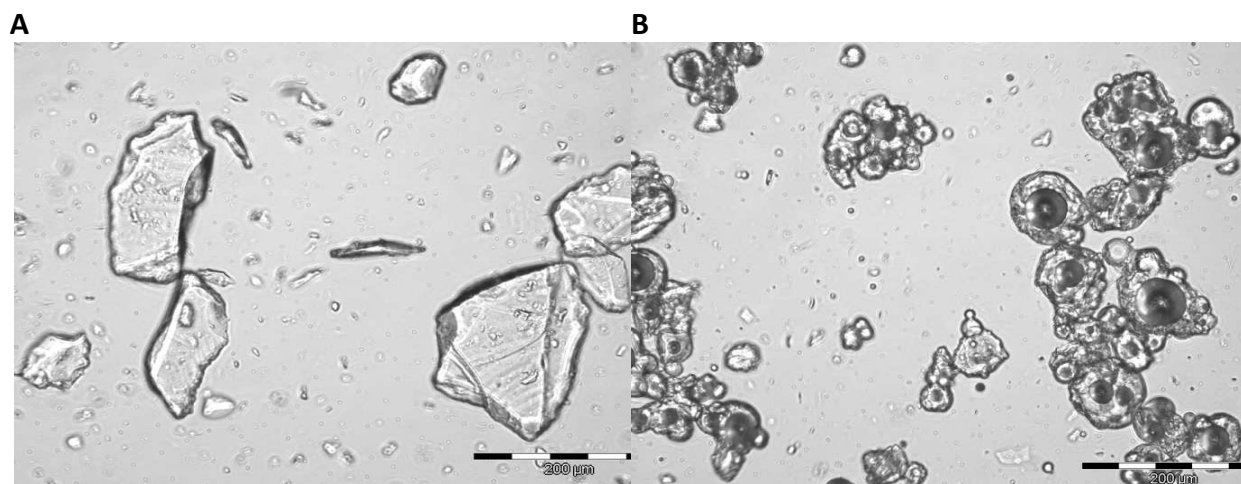


Figure 5.3. Light microscope images Polydextrose (A) and Inulin (B)

Earlier reports by Aidoo et al. (2014a) also revealed clear variations in the microstructure of inulin and polydextrose in molten chocolate. Chocolate formulations containing polydextrose showed large crystals with dense smaller particles in between the larger crystals and minimal inter-particle spaces where as formulations containing inulin revealed

large crystals with more void spaces between the crystals indicating limited particle - particle interaction strength. A combination of inulin and polydextrose in ratio of 75 and 25 % respectively, at particle sizes of 20 μm and 30 μm in our studies could therefore provide a structure where fine particles from polydextrose fill the gaps of coarser particles of both inulin and polydextrose in optimum proportions, resulting in decrease in viscosity. ANOVA showed that both PS and fat content had significant ($P \leq 0.05$) effect on Casson viscosity with significant interaction between factors. This means that the combined influence of fat and PS could be used to control the chocolate viscosity. Multiple range tests showed that Casson viscosity for all three fat levels were significantly different at the 95.0% confidence interval. The Casson viscosity at 50 μm was significantly ($P < 0.05$) higher than Casson viscosity at 20 μm and 30 μm at all fat levels. Casson viscosity for 20 μm and 30 μm were however not significantly ($P \leq 0.05$) different at all fat levels.

The Casson viscosity according to Seguire (1988) relates to pumping characteristics, filling of rough surfaces, coating properties and sensory character of body. Sugar-free chocolate manufacturers must therefore take extreme caution at the level of fat at which they produce their products as this might have dying consequences on the viscosity of the molten chocolate making it difficult for pumping and tempering procedures thereby affecting cost of production and ultimately the quality of the finished product.

5.6.1.2 Casson yield stress

The Casson yield stress is the stress required to make chocolate begin to flow. It represents the low shear-rate properties of chocolate and is affected by particle-particle interaction, the amount and specific surface area of the particles, emulsifiers and moisture (Afoakwa et al., 2007b; Aidoo et al., 2013; Aidoo et al., 2014 a,b). A major contribution to the Casson yield stress is the absolute distance between solid particles in chocolate. Results showed that the Casson yield stress and Casson viscosity followed a similar trend. The Casson yield stress increased with decrease in fat content (Fig. 5.4). This can be attributed to increased interactions between solid particles due to increase in the solids dispersed fraction at reduced fat levels thereby limiting chocolate flow. Beckett (2000) explained that the effect

of an extra 1% fat on yield value depends on the amount already present. Above fat content of 32%, there is very little change in yield value with any further additions.

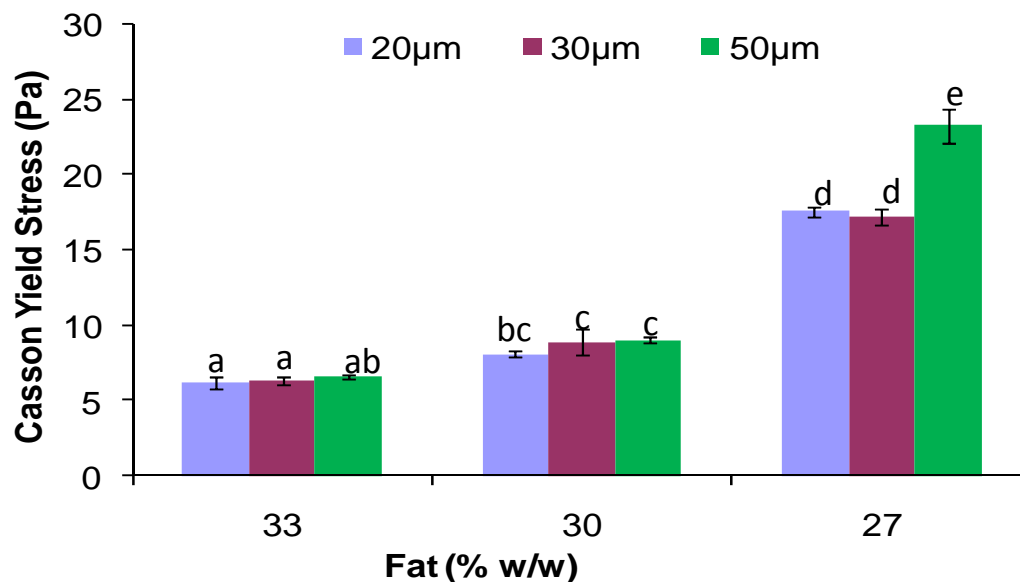


Figure 5.4 Effect of particle size on Casson yield stress of sugar-free dark chocolates with polydextrose/inulin mixtures as bulking agents at different fat contents. [samples with different letters are significantly ($P \leq 0.05$) different]

The Casson yield numbers from this work were within the range reported for dark chocolate, i.e 4 - 32 Pa. The Casson yield stress increased from 17.57 Pa to 23.23 Pa with increase in particle sizes from 20 μm to 50 μm at the 27% fat level. As mentioned earlier, the yield stress corresponds to the energy needed for chocolate to start moving at rest. Translated on microscopic scale, it relates to the strength of particle aggregates at rest. The Casson viscosity, defined at 50 s^{-1} which corresponds to high shear flow properties, reflects a structure made of particle aggregates of a certain size that is specific to the shear rate 50 s^{-1} . The aggregate size under high viscous forces (linked to high shear viscosities) and the strength of the aggregated network (related to the yield stress) seem therefore to have the same interaction origin (Servais et al., 2003), hence the linear correlation between the yield stress and viscosity.

Statistical analysis showed that both particle size and fat content had significant ($P \leq 0.05$) effect on Casson yield stress with significant ($P \leq 0.05$) interaction between both factors.

Multiple range tests showed that the Casson yield stress for all three fat levels are significantly different from each other at the 95% confidence level. The Casson yield stress for particle sizes of 20 μm and 30 μm were not significantly different from each other but significantly different from PS of 50 μm at 27% fat level. Chocolate formulations with 33% fat at 50 μm PS were however not significantly different from formulations with 30% fat at 20 μm PS for Casson yield stress. This shows the combined influence of fat and particle size on Casson yield values of the sugar-free dark chocolate and can be further explored as a means to reduce product cost since cocoa butter is an expensive ingredient.

5.6.2 Hardness

Generally, hardness increased with decrease in level of fat (Fig. 5.5). It has been reported that chocolate hardness depends on the concentration of crystallized lipid phase (cocoa butter, milk fat) as well as the solid dispersed phase (sugar crystals, milk solids, cocoa solids) (Afoakwa et al., 2007a). The concentrations of inulin/polydextrose mixtures in the 33%, 30% and 27% chocolate formulations were 47.76%, 51.76% and 55.76%, respectively (Table 5.1).

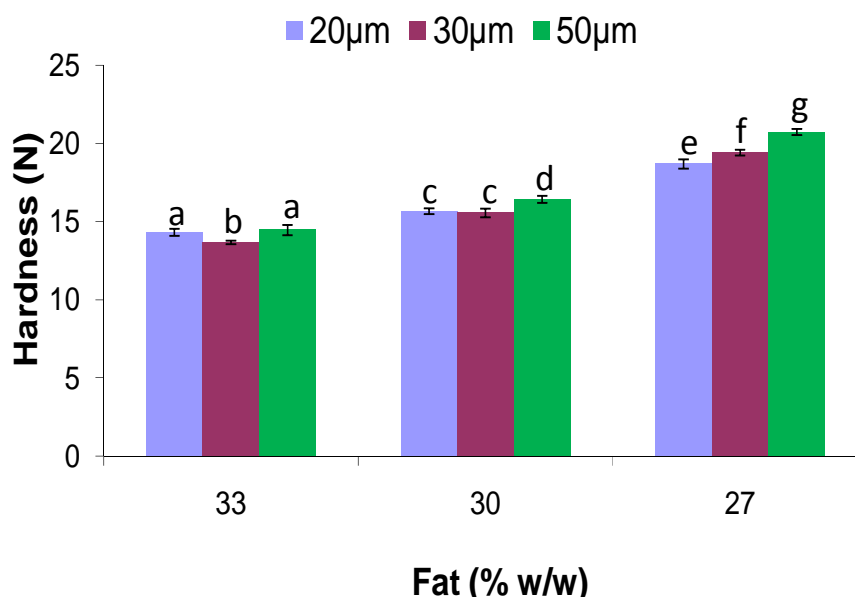


Figure 5.5 Effect of particle size on hardness of sugar-free dark chocolates with polydextrose/inulin mixtures as bulking agents at different fat contents. [samples with different letters are significantly ($P < 0.05$) different; $n = 7$]

The amount of fat was thus inversely related to the amount of bulking agent present which in turn affected the hardness of the final product since all chocolate samples were tempered. The effects of inulin and polydextrose mixtures on hardness of sugar-free chocolates have been studied by Aidoo et al. (2014a). The authors reported that high levels of the sugar substitutes lead to a hardening effect on chocolates. Chocolates with the largest particle size (50 μm) were the hardest at 30 and 27% fat contents (Fig. 5.5), recording averages of 16.42 and 20.73 N respectively. This suggests higher particle-particle interactions at larger particle sizes than at lower particle sizes at 30% and 27% fat contents. Changes in chocolate hardness with particle size, particularly at low fat contents have been reported by Do et al. (2007). Afoakwa et al. (2008) also reported the combined effect of fat content and particle size on chocolate hardness with effects less pronounced at higher fat and lecithin contents where fat coating of particles reduces inter-particle interactions and induces product softening. The authors recorded significant ($p < 0.001$) reductions in hardness with increase in lecithin content from 0.3% to 0.5%. This explains the decrease in hardness with increase in fat content at all particle sizes since higher fat content chocolates had relatively lower concentration of solids and consequently a higher lecithin to surface area ratio. Lecithin has amphiphilic (both hydrophilic and lipophilic) properties making the molecule an effective dispersant, promoting deagglomeration and wetting of clumps, inducing chocolate softening (Afoakwa et al., 2008). ANOVA showed that both PS and fat content had significant ($P \leq 0.05$) effect on hardness with significant interaction between both factors. Multiple range tests showed that the hardness values for all three fat levels were significantly different from each other. Significant ($P \leq 0.05$) increases in hardness with increase in particle size was noted at 27% fat. Samples refined to particle sizes of 20 μm and 30 μm were however not significantly different at 30% fat (Fig. 5.5). The combined effect of fat content and particle size thus have great influence on hardness of sugar-free chocolates with inulin/polydextrose mixtures as sugar replacers with effects more pronounced at lower fat contents.

5.6.3. Melting Properties

The onset temperature (T-onset), maximum peak temperature (T- max), peak width at half height (T-width) and enthalpy of melting (ΔH_{melt}) were calculated automatically after integrating the melting peaks using TA Data analysis software (TA Instruments, New Castle,

USA). Table 5.2 shows the data for the studied parameters used in studying the melting properties. T-onset corresponds to the temperature at which a specific crystal form starts to melt; T-max, that at which melting rate is greatest; T-width, an indication of how long it took (duration) a particular crystal form to melt; Peak area, the energy taken for a particular crystal form to melt. According to McFarlane (1999), all these information are related to crystal type. All the samples exhibited similar distinct single endothermic transitions between 15 °C and 55 °C similar to earlier observations by Afoakwa et al. (2008). The heat capacities c_p gradually and consistently increased to onset temperature (T-onset), and then progressively increased more rapidly until peak temperature (T-max), after which it decreased to the end temperature indicating the chocolate was completely melted.

Table 5.2. Effect of particle size and fat content on melting properties (Onset T= Onset temperature; Peak max=Maximum peak temperature; Width T= Peak width at half height; Area = Enthalpy of melting) of sugar-free dark chocolates with polydextrose/inulin mixtures as sugar replacers

Fat content (% w/w)	Particle size D ₉₀ (μm)	Melting Properties			
		Onset T (°C)	Peak max (°C)	Width T (°C)	Area (W/g)
27	20 ± 1.0	29.99 ± 0.83 ^{bcd}	33.37 ± 0.40 ^a	3.72 ± 0.34 ^a	32.26 ± 1.18 ^{bc}
	30 ± 1.0	29.14 ± 1.82 ^{abc}	33.19 ± 0.27 ^a	4.16 ± 0.69 ^a	31.50 ± 1.11 ^{ab}
	50 ± 1.0	28.08 ± 0.38 ^a	34.28 ± 0.27 ^b	5.05 ± 0.27 ^b	31.05 ± 0.76 ^a
30	20 ± 1.0	30.41 ± 0.08 ^d	33.95 ± 0.29 ^b	3.72 ± 0.07 ^a	32.80 ± 1.22 ^{cd}
	30 ± 1.0	30.22 ± 0.80 ^{cd}	34.08 ± 0.49 ^b	4.14 ± 0.76 ^a	33.60 ± 1.37 ^d
	50 ± 1.0	28.65 ± 0.35 ^a	33.97 ± 0.26 ^b	4.92 ± 0.17 ^b	33.10 ± 1.07 ^{cd}
33	20 ± 1.0	30.77 ± 1.01 ^d	33.89 ± 0.74 ^b	3.75 ± 0.66 ^a	38.98 ± 0.95 ^e
	30 ± 1.0	30.40 ± 0.81 ^d	34.13 ± 0.23 ^b	4.09 ± 0.46 ^a	40.25 ± 0.17 ^f
	50 ± 1.0	28.98 ± 1.04 ^{ab}	34.13 ± 0.43 ^b	4.74 ± 0.14 ^b	40.69 ± 0.82 ^f

Mean ± Standard deviations. [Means within same column with different letters are significantly different (P≤0.05); n=6]

Data from the DSC (Table 5.2) showed that T-onset and peak area were two parameters that were influenced by the level of fat. High level fat sugar-free chocolates recorded high T-onset. A similar trend was observed for the peak area where sugar-free chocolates with high

fat levels required much more energy to melt than chocolates with low fat levels. The highest enthalpy of 40.69 W/g was recorded for chocolates produced at 33% fat with the lowest enthalpy 31.05 W/g being recorded for chocolates produced at 27% fat.

ANOVA showed that fat content had a significant ($P \leq 0.05$) effect on T-onset, Peak area and T-max, but not on T-width. Multiple range tests showed that, for T-onset and T-max, samples with 30% and 33% fat were not significantly different from each other. All three fat levels were however significantly ($P \leq 0.05$) different from each other for Peak area.

Data from the DSC (Table 5.2) also shows the effect of PS on the melting properties of the sugar-free chocolate. Generally, T-width increased with increase in particle size whereas T-onset decreased with increase in particle size, regardless of the fat content. Increase in enthalpy of melt with increase in particle size was observed only at 33% fat. The decrease in T-onset with increase in particle size could be attributed to increase in surface area at lower particle sizes thereby limiting the amount of free fat available to initiate melting. Larger particle sizes tend to have lesser surface areas with less fat coating the surfaces, increasing the amount of free fat available. Increase in T-width with increase in particle size is an indication that chocolates with particle sizes of 50 μm might take much longer to melt than chocolates with particle sizes of 20 μm . ANOVA showed that PS had significant ($P \leq 0.05$) effect on T-onset, T-width and T-max. Multiple range tests showed that T-onset for chocolates with particle size of 50 μm was significantly lower than chocolates with particle sizes of 20 μm and 30 μm . T-width for chocolates with particle size PS of 50 μm was significantly ($P < 0.05$) higher than chocolates refined to PS of 20 μm and 30 μm at all fat levels. A significant ($P < 0.05$) interaction effect between fat content and PS was observed for enthalpy of melt (peak area) and T-max indicating the combined effect of these factors on the given parameters. Practically, although the sugar-free chocolates with the highest fat content will require more energy to complete melting, particle size had very little influence on melting properties of the sugar-free chocolate. This knowledge is important as it provides information on likely oral melting behavior with an impact on temporal components of flavor release and also oral epithelial sensation.

5.7 Conclusions

Particle size and fat content were significant factors determining rheological and mechanical properties of the sugar-free dark chocolates. The Casson viscosity increased significantly ($P < 0.05$) with decrease in fat level and also with increase in particle sizes from 30 μm to 50 μm at all fat levels. The effect of PS on rheological properties (Casson yield stress and viscosity) was more pronounced at 27% fat level. Sugar-free chocolate samples with largest particle sizes (50 μm) were the hardest at all fat levels. Chocolates with low fat levels recorded lower onset temperatures and enthalpy of melt. Regardless of the fat content, the peak width at half height increased significantly with increase in particle size from 30 μm to 50 μm . Inulin and polydextrose mixtures can be used for sugar-free chocolate manufacture with satisfactory flow, melting and mechanical properties with modifications in PS and suspending medium (cocoa butter). Particle size could thus be manipulated with the combined action of fat to control the rheological and mechanical properties of sugar-free dark chocolates, with great significance on quality control and reduction in production cost.

**Chapter 6: Functionality of inulin and polydextrose mixtures in
defining the microstructural behaviour and physical quality
characteristics of sugar-free dark chocolate**

6.1 Introduction

Bulk sweeteners are ingredients that can substitute for both the physical bulk and sweetness of sucrose. Often referred to as “sugar replacers”, bulk sweeteners are constantly being explored industrially for their importance in food applications. Several health-promoting effects have been attributed to these ingredients and thus have potential advantages over sugar as food ingredients (Aidoo et al., 2013). Chocolate is among the world’s most popularly manufactured and consumed confectionary product. Because of its sweet taste and pleasurable mouth feel, millions of tonnes of chocolate bars and fillings are produced each day in companies all over the world.

Over the past decades, food technologists in chocolate processing industries have carried out fundamental and applied research on chocolate composition and processing, in order to come up with products with improved nutrition for consumers. This is partly attributed to the high caloric value of chocolate contributed mostly by the ingredients used. Chocolate is a dense suspension consisting of sugar particles, cocoa solids, and milk powder (depending on type) dispersed in cocoa butter as a continuous phase (Afoakwa et al., 2007a; Beckett, 2009; Sokmen & Gunes, 2006). The composition of sucrose in chocolate is about 30-60% (depending on type) which confers multiple functional properties on chocolate including sweetness, particle size distribution (PSD) and mouthfeel, contributing 4 Kcal/g. The impact of sucrose on rheological properties is also important for the end product quality (Afoakwa et al., 2007b; Jeffery, 1999).

Lowering the fat content of chocolate seems the obvious choice to achieve a reduction in energy density since fat has the highest energy density of all the ingredients (9 Kcal/g). However, simple fat reduction is limited by the dominance of the volume fraction of the solids on the rheological behavior of chocolate. Consequently, edible carbohydrates with lower energy contents have been developed which are suitable for inclusion as sucrose replacers in chocolate manufacture (Aidoo et al., 2013; Aidoo et al., 2014; Afoakwa et al., 2007a; Rudolf & Stergios, 1995). The applicability and suitability of inulin and polydextrose as bulking agents in sugar-free chocolate manufacture have been reviewed (Aidoo et al., 2013). See sections 2.3.3.1 and 2.3.3.2 for detailed information on inulin and polydextrose.

6.2 Research strategy

The specific objectives of this chapter are:

1. To characterize inulin and polydextrose for effective comparison between resulting chocolate products.
2. To determine the effect of fat content on functionality of different mixtures of Inulin and polydextrose in sugar-free dark chocolate manufacture.
3. To investigate the effect of emulsifier concentration and wet conching time on flow properties of the developed sugar-free dark chocolate.

6.3 Materials and methods

6.3.1 Raw materials

For list of ingredients see section 5.3.1

6.3.2 Experimental design

Two experimental setups were conducted. The first setup varied the fat content (27, 30, 33 % w/w) and ratio of bulking agents PD: IN (0:100, 25:75, 50:50, 75:25, 100:0) simultaneously to evaluate the physicochemical properties of the sugar-free dark chocolate. Table 6.1 shows the percentage of the ingredients used to prepare the chocolates at the different fat contents. The ingredients were mixed and refined using a 3-roll refiner (Exakt SOS Apperatebau GmbH & Co. KG, Norderstedt, Germany) to 28-30 μm particle sizes. The refined chocolates were dry conched in a Buhler Elk'Oline conche (Richard Frisse GmbH Bad Salzufien, Germany) for 6h (clockwise mixing: – 1200 rpm, 60 °C, 120 min; anti-clockwise shearing: 1200 rpm, 70 °C, 120 min) with addition of 1% of extra cocoa butter to aid shearing. Lecithin and the remaining cocoa butter were added prior to wet conching at high speed (clockwise mixing: 2400rpm, 45 °C, 15 min; anti-clockwise shearing: 2400 rpm, 45 °C, 15 min) to obtain optimum mixing and liquefaction. The resulting molten chocolate obtained was kept in sealed plastic containers at ambient temperature (20-22 °C) for further analysis.

The reference contained sucrose instead of inulin and/or polydextrose as bulking agents.

The second set up varied emulsifier concentration (0.4 and 0.6 wt. %) and wet conching time (30 min and 60 min) while all other variables were kept constant. Chocolate preparation followed the same procedure as the first setup. The wet conching time was however varied: for 30 min (clockwise mixing: 2400rpm, 45 °C, 15 min; anti-clockwise shearing: 2400 rpm, 45 °C, 15 min); for 60 min (clockwise mixing: 2400rpm, 45 °C, 30 min; anti-clockwise shearing: 2400 rpm, 45 °C, 30 min). The reference contained sucrose instead of inulin/polydextrose mixtures as bulking agent.

6.3.3 Tempering procedure

The tempering procedure for both set up is as outlined in section 4.3.3

Table 6.1 Recipe for fat variations

Ingredient	Fat (% w/w)		
	33	30	27
Cocoa liquor (%)	40	38	36
Cocoa butter (%)	11.6	9.6	7.6
Bulking agent (%)	48	52	56
Lecithin (%)	0.4	0.4	0.4

6.4 Analytical methods

Characterization of bulking agents (inulin and polydextrose)

6.4.1 Density

The density of the bulking agents was calculated from mass and volume measurements obtained with a Pycometer using Isopropanol with known density at a temperature of 20 °C. Approximately 10g of the powders were added to the pycometer and then filled with isopropanol. The weights of the displaced isopropanol were determined and with its known density the volumes occupied by the powders were determined.

6.4.2 Moisture

The method is as outlined in Section 4.4.2

6.4.3 Microscopy

Microscopic images of the bulking agents polydextrose (Litesse® Two) and inulin HP were observed using a Field Emission Scanning Electron Microscope (Jeol Europe BV, Zaventem, Belgium). Because the powders were not coated, the accelerated voltage was reduced from 15 kV to 1 kV. The probe current was 8 μ A and the working distance was 20.0 mm. In addition, the light microscope (Leitz Diaplan, Pleitz Wetzlar, Germany) with Olympus Colour View camera (Olympus, Aartselaar, Belgium) was used together with temperature controlled stage Linkam PE94 (Linkam Scientific Instruments, Surrey, UK) to assess the shape of the bulking agents. The bulking agents were dispersed in vegetable oil, placed on a microscope slide and covered with a cover slip. Samples were visualized using normal light.

6.4.4 X-ray diffraction Analysis

A D8 Advance diffractometer (Bruker, Germany) (λ Cu=1.54178 Å) equipped with a Vantec (Bruker, Germany) detector, and a TTK450 low-temperature Chamber & TCU 110 Temperature control Unit (Anton Paar, Graz, Austria) connected to a water bath (Julabo, Germany) was used. The measurement time was every two minutes between 1 and 13°2 θ and also every two minutes between 15 and 27°2 θ . Short and long spacings were determined using the Bragg law: $n\lambda=2d\sin\theta$, where n is an integer, λ is the wavelength, d is the spacing between the planes in the atomic lattice, and θ is the angle between the incident ray and the scattering planes.

6.4.5 Melting properties

The thermal behaviour of bulking agents: onset temperature (T_{onset}), peak temperature (T_{max}), offset temperature (T_{offset}) and enthalpy of melting (ΔH_{melt}) were measured and calculated automatically using 2010 Differential Scanning Calorimeter (TA Instruments, New Castle, USA) equipped with a thermal analysis software using an empty aluminum pan (TA Instruments, New Castle, USA) as reference. Approximately 5 mg of the bulking agent was loaded into aluminium pans and sealed with lids using a sample press. The time-temperature program had the following steps: 1. Equilibrate at 20 °C, 2. Isothermal for 3min, 3. Ramp 5 °C/min to 195 °C, 4. Isothermal for 1min, 5. Ramp 5 °C/min to 20 °C and 6. Isothermal for 3min 7. Ramp 5 °C/min to 195 °C.

Functionality of bulking agents in chocolate at different fat contents

6.4.6 Rheological properties

The method is as outlined in Section 4.4.1

6.4.7 Texture measurements (Hardness)

The method is as outlined in Section 4.4.4

6.4.8 Colour

The method is as outlined in Section 4.4.5

6.4.9 Moisture

The method is as outlined in Section 4.4.2

6.4.10 Melting properties

The method is as outlined in Section 5.4.4

6.4.11 Microscopy

The method is as outlined in Section 4.4.6. Samples were visualized using normal light.

6.5 Statistical analysis

Analytical data obtained were processed into tables and graphs using Microsoft Excel (Microsoft Corporation, USA). Statistical analysis of results was conducted with Statsgraphics Centurion XV (Graphics Software System, STCC, Inc., Rockville, USA) using analysis of variance (ANOVA) with the level of significance set at $P < 0.05$. Multiple range tests (Tukey's honestly significant difference) were used to determine differences in means. All experiments were conducted in triplicates except otherwise stated.

6.6 Results and discussion

6.6.1 Characterization of bulking agents

Inulin, polydextrose and sucrose were characterized physico-chemically in order to gain better understanding of their functionality in chocolate and to explain possible differences in physical quality characteristics that will occur during sugar-free dark chocolate manufacture compared to the reference chocolate made with sucrose. These differences might have negative or positive impact on the final product quality.

6.6.1.1 Physicochemical properties of bulking agents

Moisture and density measurements of the bulking agents are shown in Table 6.2. Polydextrose and inulin recorded higher moisture contents of 2.10g/100g and 2.52g/100g respectively as compared to sucrose (0.08g/100g). This can be attributed to their hygroscopic nature which results in absorption of water molecules around the sugar particles, causing undesirable changes to flow behaviour of chocolate during processing (Lucisana et al., 2006). Inulin recorded the lowest density (1.31 g/cm³), followed by polydextrose (1.46 g/cm³) and sucrose (1.59 g/cm³).

Table 6.2 Physical properties of bulking agents

Bulking Agents	Moisture (g/100g)	Density (g/cm ³)
Sucrose	0.08 ± 0.01	1.59 ± 0.01
Polydextrose	2.10 ± 0.16	1.46 ± 0.01
Inulin	2.52 ± 0.03	1.31 ± 0.01

mean values ± standard deviations from triplicate analysis

Lower particle densities according to Sokmen and Gunes (2006) result in a higher particle volume fraction when replacements are done on weight basis. This might have a major impact on particle collision and aggregation in a colloidal suspension such as chocolate (Sokmen and Gunes, 2006).

6.6.1.2 Microstructure of bulking agents

Microscopic images of the different bulking agents are shown in Figure 6.1. Images from the light microscope (left) revealed their shape and particle variation while the scanning electron microscope (right) revealed their microstructure. The light microscope revealed that particles of sucrose and polydextrose are non-spherical and seem to have similar observable features of being angular and dense with variable irregular sizes and shapes (Figure 6.1A & B left). However, a critical observation showed that the particles of polydextrose are bigger and broader, often occurring singly while those of sucrose have several tiny particles attached to bigger ones. Inulin particles were more rounded at the edges (spherical), porous and had almost regular sizes (Figure 6.1 C left). The black spots noted on the images ((C) left) are air spaces in the particles reflecting its porous nature. The rough surface shown by pre-broken sucrose (Figure 6.1 A right) suggests a crystalline structure while the smooth surface shown by polydextrose (Figure 6.1 B right) suggests an amorphous material. Inulin also showed a smooth surface but had some rough edges. This suggests an amorphous material with some crystalline regions and pores. According to Do et al. (2007), non-spherical particles such as sucrose and polydextrose lead to a poorer packing efficiency in suspensions, negatively affecting the maximum particle volume fraction and viscosity.

The amorphous nature of inulin and polydextrose were confirmed by XRD analysis. As a result of differences between the amorphous and crystalline arrangements of polymer chains, the X-ray diffraction patterns of the two phases are very different. The amorphous phase contains no long-range order, meaning that there are no regular crystalline planes to diffract X-rays. Thus the incident X-rays were scattered randomly and there were no sharp peaks in the diffraction pattern as observed for polydextrose and inulin (Figure 6.2). In the crystalline phase, the repeating lamellar chains provide a regular structure, and thus the diffraction pattern contains sharp, prominent signature peaks, as observed for sucrose (Figure 6.2), the position of which depends on the exact spacing between chains.

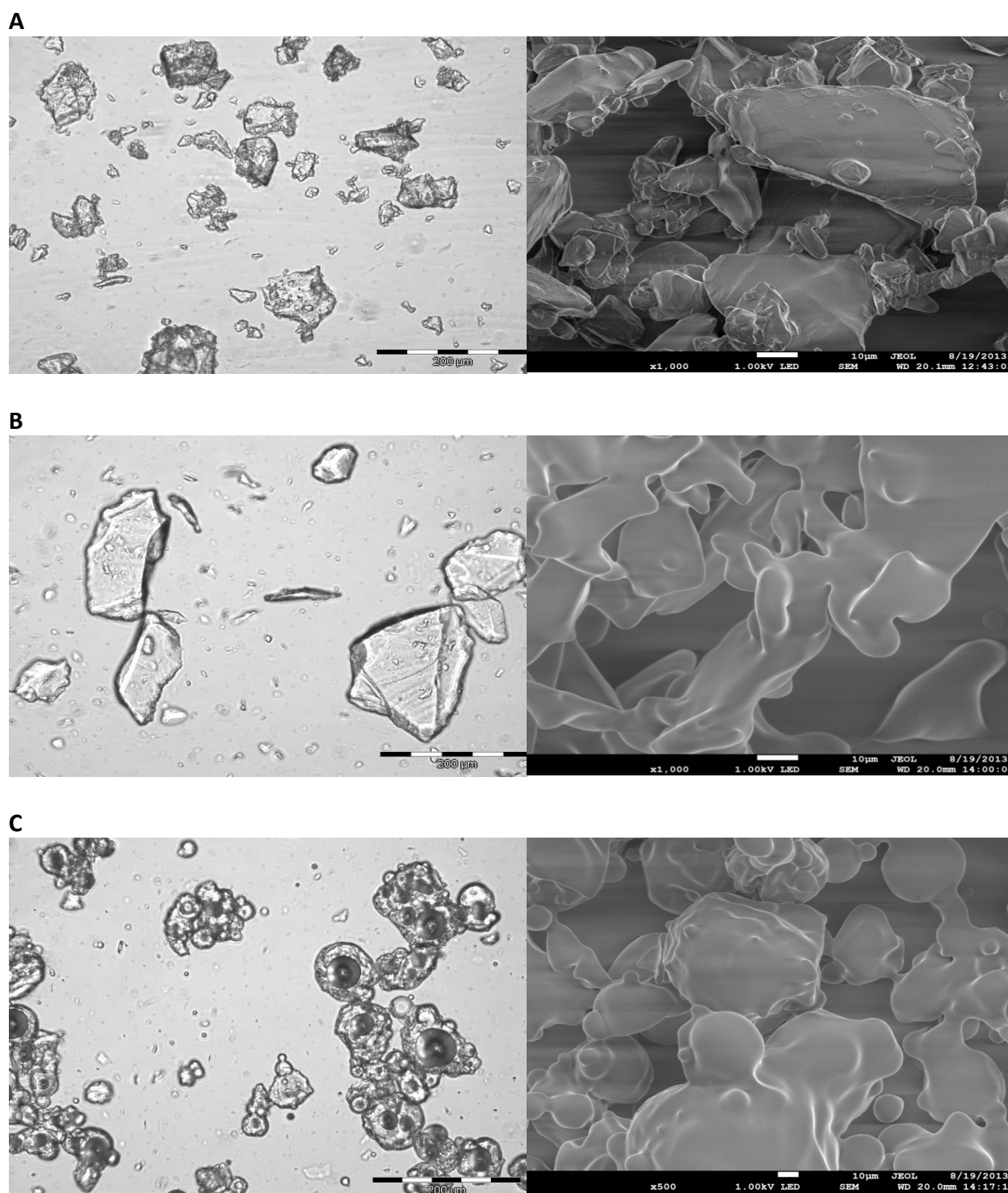


Figure 6.1. Light microscope images (left) and scanning electron microscope images (right) of (A) Sucrose (B) Polydextrose and (C) Inulin

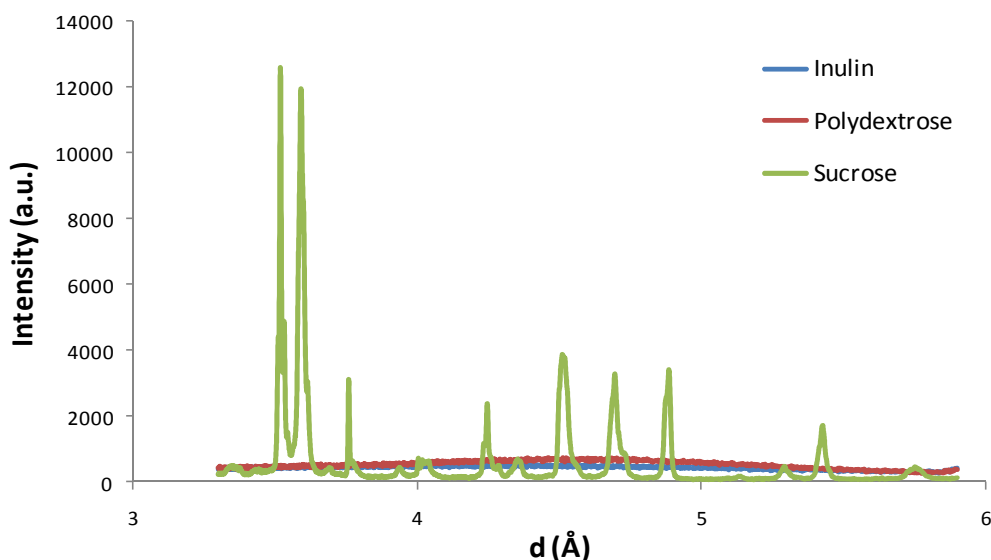


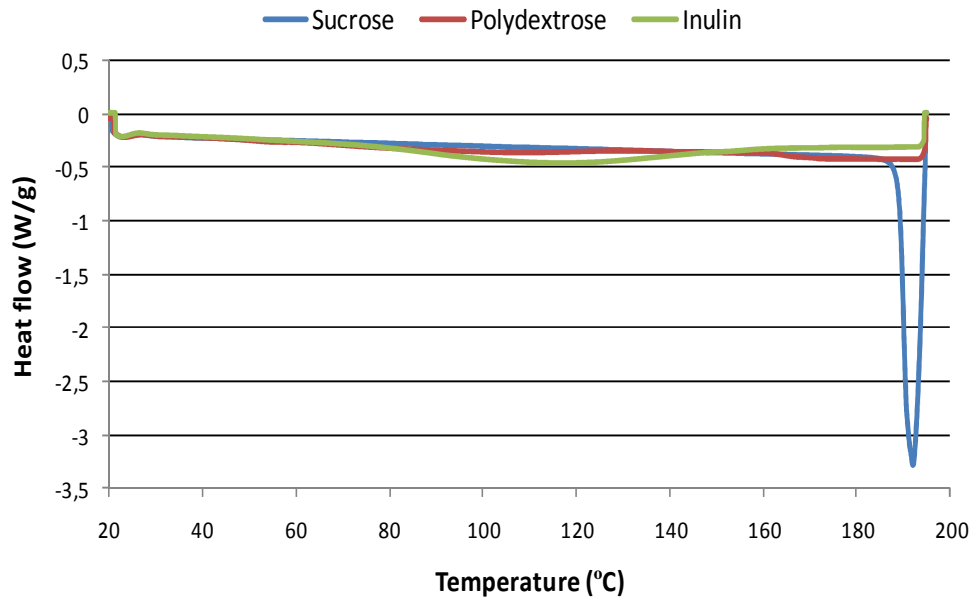
Figure 6.2 X-ray diffraction patterns of sucrose, polydextrose and inulin

6.6.1.3 Thermal behaviour of bulking agents

Thermal analysis techniques such as differential scanning calorimetry (DSC) can also be used to determine the degree of crystallinity of a sample. The thermal transitions for the three bulking agents are shown in Figure 6.3 a. A sharp distinct melting peak was observed for sucrose, starting from 189 °C (Tonset) with the greatest melting rate occurring at 191 °C (Tmax) and absorbing 136 J/g (ΔH_{melt}) of latent heat. No clear melting peak was observed for either polydextrose or inulin (Figure 6.3 a). A much detailed thermograph of inulin and polydextrose (Figure 6.3 b) however revealed a much broader profile for inulin than for polydextrose with inulin showing a shallow and broader peak (Figure 6.3 a, b). The presence of a melting peak in sucrose confirms its crystalline structure while the absence of a peak in polydextrose shows that it has mainly amorphous regions. The broad but visible melting peak of inulin (Figure 6.3 b) also suggests a polymer with both amorphous and crystalline regions. Thus Inulin may have an average degree of polymerisation high enough to possess sufficient crystalline thickness with high melting point (Hebette et al., 1998). Semicrystalline polymers according to Weiss and Ober (1990) have true melting temperatures at which the ordered regions break up and become disordered. In contrast, the amorphous regions soften over a relatively wide temperature range (always lower than melting temperatures) known as the glass transition (T_g). Fully amorphous polymers do not exhibit melting temperatures,

but all polymers exhibit T_g . Above these temperatures, polymers are liquids (Weiss and Ober, 1990).

a.



b.

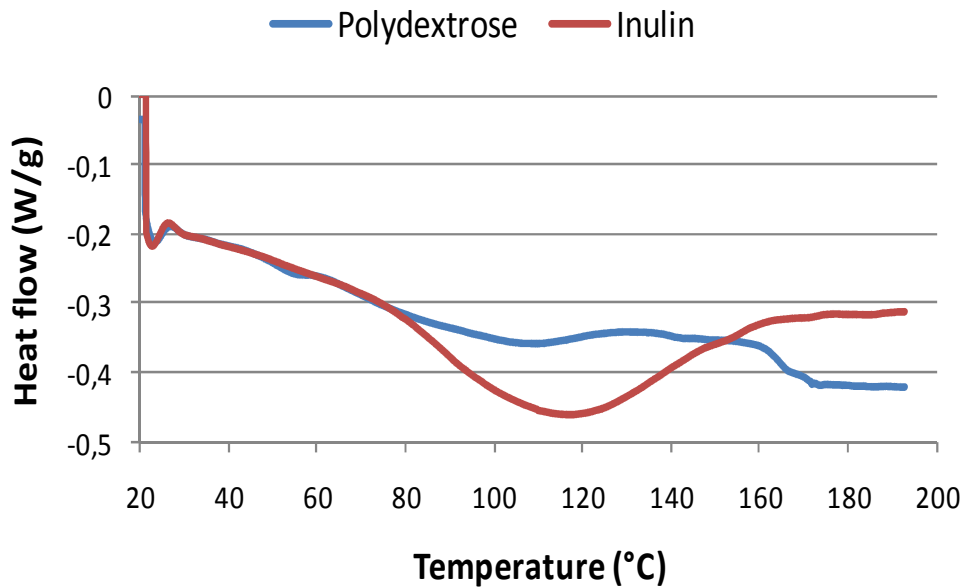


Figure 6.3 Thermal transitions for a. sucrose, polydextrose and inulin b. polydextrose and inulin

Glass transition occurs in amorphous materials, whose chains are not arranged in ordered crystals even though they are in solid state (Abbas et al., 2010). Some crystalline polymers such as sucrose has amorphous portions and exhibits both glass transition and melting behaviours. The amorphous portion undergoes glass transition only while the crystalline portion undergoes melting. All bulking agents underwent glass transition (Figure 6.4) upon cooling at constant cooling rate - the best measure for glass transition temperature (T_g) for the characterization of polymers. Sucrose had the lowest T_g ($T_{g,onset}$ 60.81; $T_{g,mid}$ 64.4; $T_{g,offset}$ 128.9) because it has the lowest molecular weight (342 g/mol). Polydextrose and inulin recorded higher T_g 's [$(T_{g,onset}$ 93.9; $T_{g,mid}$ 101.3; $T_{g,offset}$ 133.2) and ($T_{g,onset}$ 128.9; $T_{g,mid}$ 109.9; $T_{g,offset}$ 137.0), respectively] because of their high molecular weights.

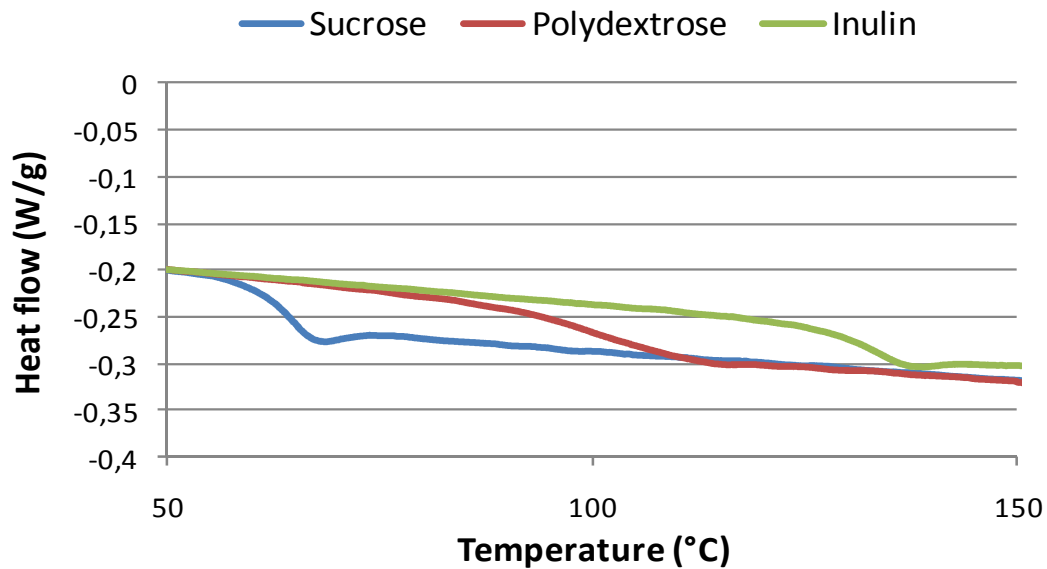


Figure 6.4 Glass transition for the bulking agents sucrose, polydextrose and inulin

6.6.2 Functionality of inulin and polydextrose on physicochemical properties of sugar-free dark chocolates.

6.6.2.1 Rheological properties

Materials with both solid and fluid features are often characterised experimentally by rheology in order to give a practical understanding of their mechanical behaviour when subjected to different forms of stress (Goncalves and Lannes, 2010). Food technologists use information on the flow properties of materials as a quality control tool to ensure the

consistency of products and to reduce batch variations. Rheological properties of the different samples of chocolates produced were applied to the Casson model (Eq 1.2).

6.6.2.1.1 Casson viscosity

Like many water-in-oil suspensions, molten chocolate is a non-Newtonian fluid with shear thinning behaviour (Beckett 2000). Chocolate viscosity is particularly important because it is desirable to make chocolate flow as optimally as possible at any fat content for pumping and moulding purposes. The viscosities of all chocolate samples produced at 33% fat were within the range of 2.1 to 3.9 Pa.s, as reported by Aeschlimann and Beckett (2000). Chocolate samples produced at 30% and 27% fat contents however exhibited significantly ($P < 0.05$) higher viscosities indicating an increase in Casson viscosity with a decrease in chocolate fat content. This was expected as more fat facilitates the movement of particles past each other thereby increasing lubrication and ultimately decreasing viscosity. Figure 6.5 shows the effect of bulking agent concentration on Casson viscosity at varying fat levels.

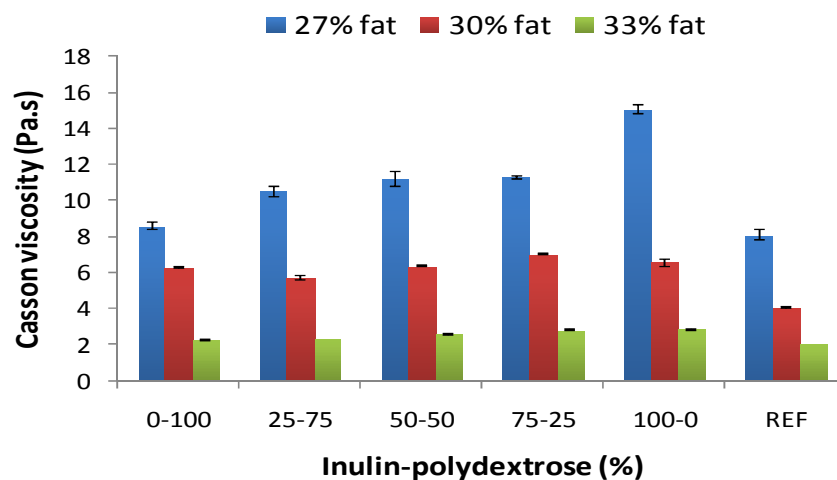


Figure 6.5 Effect of bulking agent concentration on Casson viscosity at different fat contents

It was observed that, as the concentration of polydextrose was decreased while increasing inulin concentration, the Casson viscosity increased regardless of the fat level. This confirms studies by Aidoo et al. (2014a) who reported increase in Casson viscosity with increase in inulin concentration in sugar-free dark chocolates. The high Casson viscosity associated with increased inulin concentrations can partly be attributed to the lower density of inulin

(1.31g/cm³) compared to that of sucrose and polydextrose (Table 6.2) resulting in a higher particle volume fraction in the chocolate matrix. Sucrose replacement by the bulking agents was done on weight basis therefore chocolates with increasing inulin concentration had more solids per volume and thus increased surface area. This reduces the amount of fat available for flow, as more fat is needed to coat the increased surface area of the particles thereby causing an increase in viscosity.

Statistical analysis showed that fat content had a significant effect ($P < 0.05$) on Casson viscosity. Multiple range tests showed that samples at the different fat levels (27%, 30% and 33%) were significantly different from each other at the 95.0% confidence level. The Casson viscosity for all sugar-free chocolates processed at 30% and 33% fat were significantly ($P < 0.05$) higher than their reference samples. Casson viscosity for formulation with 100% polydextrose at 27% fat was however not significantly ($P > 0.05$) different from that of the reference at 27% fat. The effect of bulking agents concentration on Casson viscosity was more pronounced at lower fat contents (27% - 30%) and processing at such low fat content might negatively impact processing variables such as pumping, moulding and sensory properties especially for sugar-free chocolates containing high levels of inulin.

6.6.2.1.2 Casson yield stress

Similar to plastic viscosity, yield stress is dependent on fat content in chocolate with much emphasis on the amount of small particles and inter-particle contacts in chocolate (Servais et al., 2002). It considers the frictional and chemical forces around and between the solid particles in the fat matrix. The yield stress for all samples fell within the range 4-32 Pa, as reported for dark chocolates (Aeschlimann and Beckett, 2000). The Casson yield stress increased with increase in polydextrose concentration for chocolates produced at 33% and 30% fat contents. No clear pattern was however observed with regards to bulk mixture concentration effect on Casson yield stress for chocolates produced at 27% fat (Figure 6.6).

Aidoo et al. (2014a) also reported increase in Casson yield stress with increase in polydextrose concentration. The authors associated this with the PSD of the polydextrose which showed a unimodal distribution compared to inulin which showed bimodal and

trimodal distributions. PSD of unimodal distributions are known to have greater influence on yield stress due to their smaller particle sizes resulting in increased surface areas of particles and inter-particle interactions. In this study, increasing polydextrose content caused a substantial decrease in PSD parameters; [D43] (mean particle diameter), [D32] (sauter mean diameter), [D10], [D50] and [D90] (10%, 50%, and 90% of all particles finer than this size, respectively). As particle size decreases, the amount of inter-particle contact points and bonds increases, causing higher yield values as relatively higher stresses are needed to disrupt the network formed and to initiate flow (Beckett, 2000, Servais et al., 2002 and Lucisana et al., 2006).

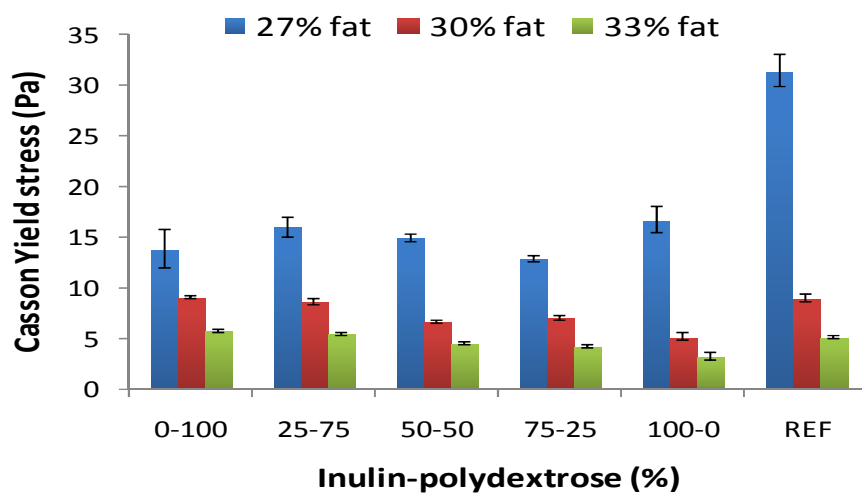


Figure 6.6 Effect of bulking agent concentration on Casson yield stress at different fat contents

Fat content had a significant effect ($P < 0.05$) on Casson yield stress. Multiple range tests showed that Casson yield stress at all three fat concentrations (27%, 30% and 33%) were significantly different at the 95.0% confidence level. All sugar-free chocolates processed at 27% fat were significantly ($P < 0.05$) different from their reference sample which recorded a significantly higher ($P < 0.05$) Casson yield stress. At 30% fat, chocolate formulations with 100% PD and 75% PD : 25% IN were statistically not different from the reference sample. Samples with 75% PD : 25% IN also did not differ significantly from the reference sample at 33% fat. This confirms studies by Aidoo et al. (2014a) who reported mixtures of 75% polydextrose and 25% inulin as optimum concentrations for their sugar-free dark chocolate manufacture. The Casson yield stress of chocolate formulated with the optimum blend (75%

PD : 25% IN) of the sugar replacers in their study was also not significantly ($P>0.05$) different from their reference sample made with sucrose (Aidoo et al., 2014a). Processing at high fat contents (30% - 33%) and high polydextrose concentrations (>75%) thus do not lead to significant ($P<0.05$) changes in Casson yield stress.

6.6.2.2 Texture

Chocolate texture is a complex physical quality parameter that can be explained in many different ways by ingredient composition, rheological properties, particle size distribution and microstructure (Afoakwa et al., 2008b). Food technologists are often concerned with chocolate textural properties such as smoothness, ease of melt in mouth and hardness of which the latter is of significant interest to this study. Textural properties of chocolate samples expressed as hardness (maximum force) are presented in Figure 6.7.

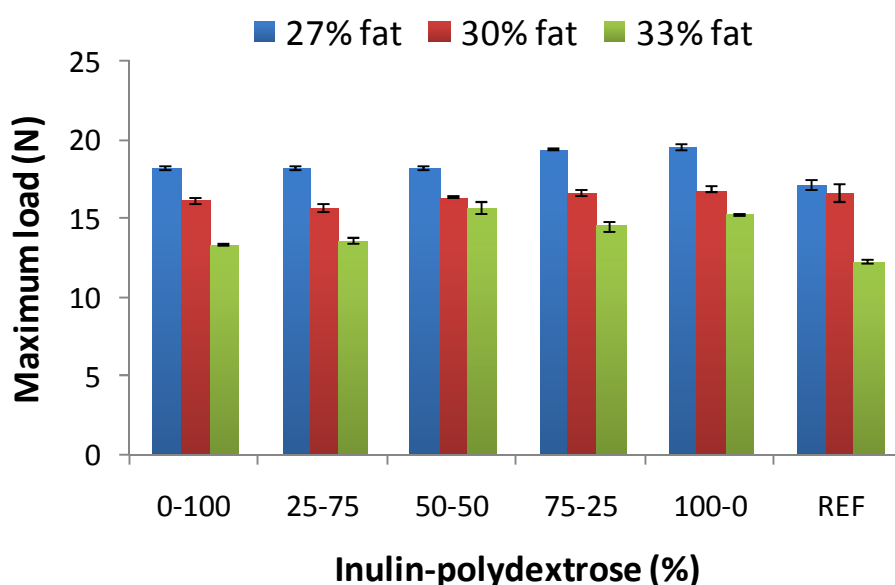


Figure 6.7 Effect of bulking agent concentration on hardness of chocolate at different fat content

Increasing the fat content resulted in decrease in hardness at all bulking agent concentrations. This is as a result of the total non-solid content being higher in chocolates containing 33% fat than 27% fat which reduces particle-particle interaction resulting in a less

dense network structure (Beckett, 1999). The hardest chocolate was chocolate formulation with 100% inulin at 27% fat content (19.62 N) and chocolate formulation with 100% polydextrose at 33% fat was the softest (13.43 N).

ANOVA showed that fat content and bulk concentration had significant ($P < 0.05$) effect on the hardness of the chocolate samples. Multiple range tests showed that hardness at all three fat concentrations (27%, 30% and 33%) were significantly ($P < 0.05$) different at the 95.0% confidence level. The hardness values for all sugar-free chocolates processed at 27% and 33% fat were significantly higher than their reference samples. Hardness of chocolates containing 100% PD, 25% PD:75% IN, 50% PD:50% IN and 100% IN were however not significantly ($P < 0.05$) different from the reference at 30% fat. The increase in hardness caused by the sugar replacers can be beneficial in applications such as ice creams, desserts and biscuits.

6.6.2.3 Moisture

According to Chevalley (1999), the moisture content of standard chocolate ranges between 0.5-1.5%. This normally occurs in the cocoa solids for dark chocolates. Moisture affects chocolate flow significantly; increasing friction and apparent viscosity. The effect of fat content and bulking agent concentration on chocolates moisture content was not trendy (Figure 6.8). Anova showed that fat content had no significant effect on moisture levels of the sugar-free chocolates. The moisture content of all sugar-free chocolates processed at 27% and 33% fat content were significantly ($P < 0.05$) higher than their reference samples. Samples containing 100% IN, 75% PD:25% IN, and 25% PD:75% IN were however not significantly ($P < 0.05$) different from the reference at 30% fat. Earlier studies on optimization of the bulking agents (chapter 4) also reported higher moisture contents for sugar substituted chocolates compared to the reference sample made with sucrose. The moisture contents of the sugar-free chocolates produced in chapter 4 were however not as high as the moisture contents reported in this study. This could have been as a result of differences in the moisture levels of the starting materials for both experiments. Inulin and polydextrose are hygroscopic materials and as such can easily pick up moisture if not properly stored. The differences in the quantity of refined chocolate that was conched, coupled with the

differences in the conching process for both experiments may also account for the differences in moisture content.

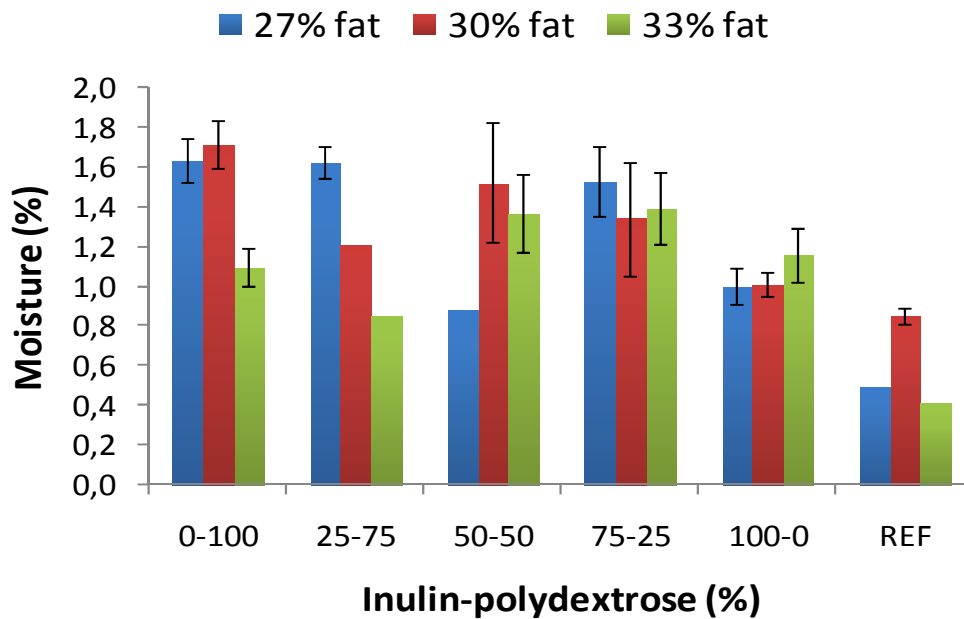


Figure 6.8 Effect of bulking agent concentration on moisture of chocolate at different fat content

The high moisture content of sucrose-free chocolate samples could be a contributing factor to the significantly high viscosities of the sugar substituted chocolates compared to the reference samples. Excess moisture surrounds the surfaces of solid particles limiting their effective coating by cocoa butter (Lucisana et al., 2006). The reduced intimate association between solid particles and cocoa butter causes the formation of gritty lumps, increasing friction and ultimately viscosity.

6.6.2.4 Colour

Colour is one of the most important commercial attributes of chocolate. Although often perceived by the human eye, instrumental analysis using L^* values (lightness-darkness (100-0)) is used by the industry and food technologists to compare the colour of many products. The higher the concentration of inulin in sucrose-free chocolates the lower the L^* value

(increased darkness) while the higher the concentration of polydextrose the higher the L^* value (decreased darkness) (Figure 6.9).

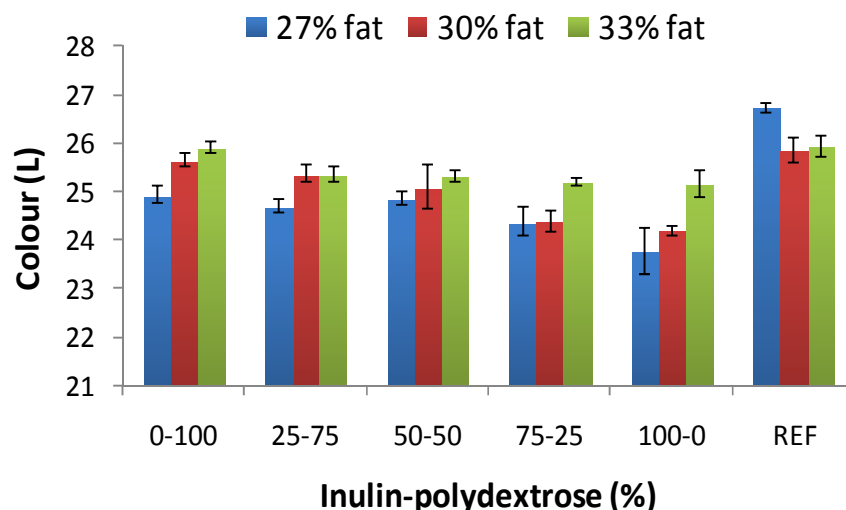


Figure 6.9 Effect of bulking agent concentration on colour (L^* value) of chocolate at different fat content

Sucrose replacement with inulin and polydextrose in chocolate resulted in darker chocolates at all fat contents with the exception of chocolate formulations containing 100% PD which were significantly ($p>0.05$) not different from their reference samples at 30% and 33% fat content. Statistical evaluation showed that fat content and bulk concentration had significant effects ($P<0.05$) on colour of chocolate samples. Shah et al. (2010) reported similar results where sucrose replacement with polydextrose and inulin in milk chocolates also resulted in darker chocolates. The authors attributed this to compositional differences. The increase in darkness of sucrose-free dark chocolates can be attributed to the roughness caused by changes in the surface properties as a result of the presence of polydextrose and inulin in the chocolate composition (Briones et al., 2006). According to Afoakwa et al. (2008b), chocolate colour appears lighter with the presence of fine particle sizes. This probably explains why the reference chocolates and sugar-free chocolates with high polydextrose concentration recorded higher L^* values (less dark) since PSD analysis revealed smaller particle sizes for such formulations.

6.6.2.5 Melting properties

In order to understand the nature or type of fat crystals formed in chocolates, their melting profile is often recorded. The temperature at which specific crystals start and end melting corresponds to the onset (Tonset) and offset temperature (Toffset), respectively (Afoakwa et al., 2008a). The amount of energy needed to complete the melting is the enthalpy (ΔH_{melt}) and the temperature at which melting rate is the highest is the maximum temperature (Tmax). The melting parameters are shown in Table 6.3.

Table 6.3 Melting properties of sugar-free dark chocolates

Fat (%)	Bulking agents conc. (%)	Onset T (°C)	Maximum T (°C)	Offset T(°C)	Energy (W/g)
27	Sucrose (100)	30.5 ± 1.4 ^a	35.1 ± 0.5 ^a	37.6 ± 0.7 ^a	29.0 ± 3.6 ^a
	PD:IN (100:0)	32.1 ± 0.4 ^a	36.0 ± 0.5 ^a	38.3 ± 0.6 ^a	30.6 ± 0.8 ^a
	PD:IN (75:25)	31.2 ± 1.1 ^a	35.6 ± 0.9 ^a	38.4 ± 0.5 ^a	27.7 ± 1.0 ^a
	PD:IN (50:50)	31.1 ± 0.8 ^a	35.2 ± 0.3 ^a	37.5 ± 0.8 ^a	29.0 ± 0.7 ^a
	PD:IN (25:75)	32.4 ± 0.4 ^a	35.6 ± 0.3 ^a	38.2 ± 0.2 ^a	26.9 ± 0.2 ^a
	PD:IN (0:100)	31.2 ± 0.2 ^a	35.6 ± 0.4 ^a	38.5 ± 0.5 ^a	29.0 ± 1.0 ^a
30	Sucrose (100)	30.5 ± 0.3 ^a	35.1 ± 0.3 ^a	37.6 ± 0.2 ^a	36.8 ± 0.3 ^a
	PD:IN (100:0)	30.7 ± 1.2 ^a	36.5 ± 0.5 ^b	39.5 ± 0.5 ^b	36.7 ± 0.1 ^a
	PD:IN (75:25)	31.8 ± 0.4 ^a	35.0 ± 0.1 ^a	37.5 ± 0.4 ^a	36.4 ± 0.6 ^a
	PD:IN (50:50)	31.1 ± 0.9 ^a	35.4 ± 0.4 ^a	37.6 ± 0.4 ^a	34.4 ± 0.6 ^b
	PD:IN (25:75)	30.5 ± 0.4 ^a	35.2 ± 0.0 ^a	37.7 ± 0.1 ^a	34.5 ± 0.1 ^b
	PD:IN (0:100)	32.2 ± 0.1 ^a	36.8 ± 0.5 ^b	39.2 ± 0.4 ^b	34.8 ± 0.2 ^b
33	Sucrose (100)	32.6 ± 0.6 ^{bc}	34.9 ± 0.3 ^a	37.2 ± 0.2 ^a	41.4 ± 1.8 ^b
	PD:IN (100:0)	31.6 ± 0.6 ^{ab}	36.8 ± 0.1 ^b	39.6 ± 0.2 ^b	40.0 ± 1.7 ^b
	PD:IN (75:25)	31.2 ± 0.2 ^{ab}	34.7 ± 0.3 ^a	37.3 ± 0.2 ^{ab}	40.5 ± 0.6 ^a
	PD:IN (50:50)	30.4 ± 0.6 ^a	35.3 ± 0.5 ^a	38.2 ± 0.6 ^b	37.0 ± 0.3 ^b
	PD:IN (25:75)	33.5 ± 0.6 ^c	36.7 ± 0.3 ^b	39.4 ± 0.3 ^{ab}	40.5 ± 0.3 ^{ab}
	PD:IN (0:100)	30.3 ± 0.6 ^a	35.4 ± 0.5 ^a	38.4 ± 1.2 ^a	38.9 ± 0.5 ^b

Means ± standard deviations from triplicate analysis. Means within same column with different letters are significantly different ($P \leq 0.05$) at each fat content.

All samples exhibited a similar distinct single endothermic transition in the range of 30.3 °C to 39.6 °C as expected for well-tempered chocolate. Increasing the fat content from 27% to 33% caused no significant ($P>0.05$) changes in onset, maximum and offset temperatures. Increasing fat content however resulted in significant increases in the melting enthalpy (Table 6.3). At 27% fat, none of the sugar-free chocolates were significantly different from the reference sample for all the melting properties. The maximum and offset temperatures for samples with 100% PD and 100% IN were not significantly different from each other at 30% fat but significantly ($P<0.05$) different from the reference. A similar observation was recorded for enthalpy of melting at 33% fat but both samples (100% PD and 100% IN) were not significantly different from the reference for melting enthalpy at 33% fat. Overall, varying the concentrations of the bulk ingredients showed very little influence on the chocolate melting properties.

6.6.3 Process optimization for sugar-free dark chocolate manufacture

To attain optimum flow and physical properties of sugar-free dark chocolates produced with inulin and polydextrose as bulking agents and sweetened with stevia rebaudiana extract, further optimization regarding formulation and processing was explored. Sugar-free dark chocolate with polydextrose and inulin concentrations of PD:IN 75%: 25% (determined as optimum concentrations) at 30% fat was used for this investigation. The effects of lecithin concentration (LEC (%)) and wet conching time (WC (min)) were evaluated. The reference sample contained sucrose and 0.4% lecithin concentration.

6.6.3.1 Rheological properties

The functionality of emulsifiers is associated with their surface-active properties in colloidal dispersions. A decrease in viscosity and yield stress in conventional chocolates at lecithin concentrations below 0.4% or 0.5% (Aeschlimann and Beckett 2000, Servais et al., 2002 and Schantz and Rohm 2005) have been reported.

6.6.3.1.1 Casson viscosity

Chocolate viscosity as reviewed by Goncalves and Lannes (2010) can be affected by fat content, ingredient composition, particle size distribution, moisture content, emulsifier content and conching time. The results showed that increasing the lecithin concentration and wet conching time resulted in a decrease in the Casson viscosity (Figure 6.10). The decrease in Casson viscosity with increase in wet conching time was much pronounced at 0.6% lecithin concentration than at 0.4% lecithin concentration.

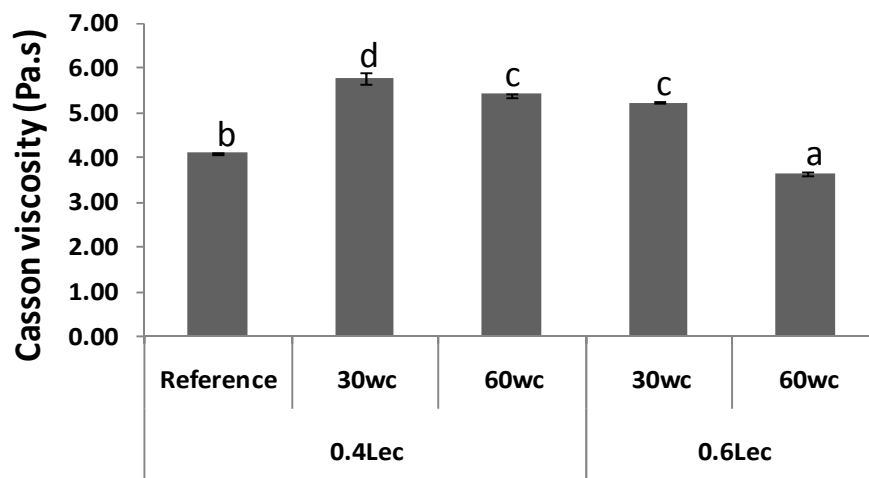


Figure 6.10 Effects of wet conching time and lecithin concentration on Casson viscosity of sugar-free dark chocolate [samples with different letters are significantly ($P < 0.05$) different]

Statistical analysis showed significant reduction in Casson viscosity with increase in wet conching time from 30 to 60 min at both lecithin concentrations. Increasing lecithin concentration from 0.4% to 0.6% while maintaining the same wet conching time also resulted in significant reduction in the Casson viscosity. Casson viscosity values for all samples were however significantly different from the reference sample. Chocolate samples containing 0.6% lecithin and wet conched for 60min recorded significantly lower Casson viscosity than the reference sample. The Casson viscosity for the sample containing 0.4% lecithin and wet conched for 60 min was however not significantly different from the sample containing 0.6% lecithin and wet conched for 30min. This shows that increasing wet conching time from 30 min to 60 min has the same effect on the Casson viscosity as increasing the lecithin concentration from 0.4% to 0.6%. Increasing the concentration of lecithin from 0.4% to 0.6% promoted adequate adsorption of emulsifier around solid particle

surfaces, thereby lowering the interfacial tension between the dispersed and the continuous phase which led to the observed reduction in Casson viscosity. Moreover, increasing wet conching time from 30 to 60 min enhanced the effective coating of solid particles with cocoa butter, thus further lowering the plastic viscosity.

6.6.3.1.2 Casson yield stress

Yield stress is a material property corresponding to the energy required to induce chocolate flow, relating to inter-particle interactions at rest (Shah et al., 2010). The effect of wet conching time and lecithin concentration on Casson yield stress of the sucrose free dark chocolate is shown in Figure 6.11. Increasing the lecithin concentration from 0.4% to 0.6% had no significant effect on the Casson yield stress at both wet conching times. Increasing the wet conching time from 30 to 60 min however resulted in a significant decrease in the Casson yield stress at both lecithin concentrations. This can be attributed to increased homogeneity following longer wet conching times. Sugar-free chocolates wet conched for 30 min were not significantly different from the reference sample regardless of the lecithin concentration.

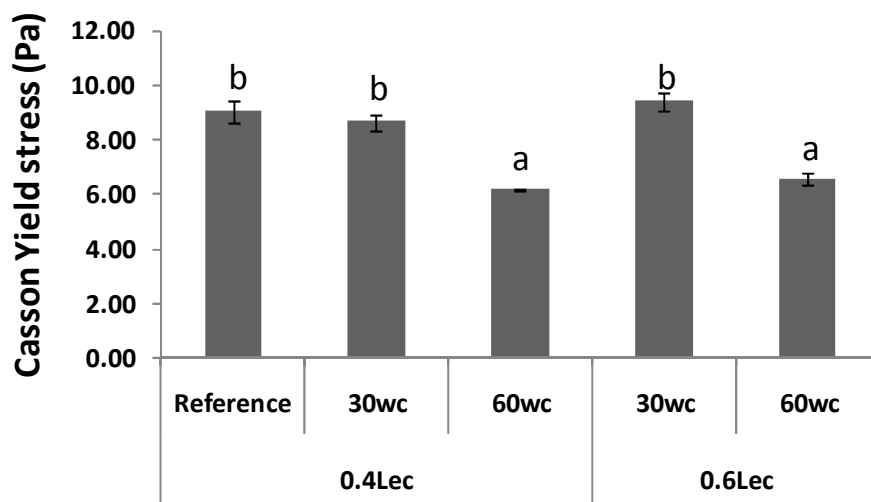


Figure 6.11 Effects of wet conching time and lecithin concentration on Casson yield stress of sugar-free dark chocolate [samples with different letters are significantly ($P < 0.05$) different]

It is known that, upon adding lecithin to chocolate, the yield value decreases until a critical lecithin concentration above which the yield value will again increase (Beckett, 2009). Our results therefore prove that lecithin can be added to the sugar-free dark chocolate up to concentrations of 0.6% without any increase in the Casson yield stress. The fact that yield value increases again above a specific concentration of lecithin may be due to the formation of bilayers around the solid particles or to the formation of lecithin micelles, both reducing the effectiveness of the emulsifier (Beckett, 2009).

6.6.3.2 Texture

The texture of chocolate is seen as a combination of its physical structure, mechanical and surface properties (Minifie, 1989). The most widely used indicator for chocolate texture in the formation of a stable fat crystal network is hardness. Other indicators utilized according to Afoakwa et al., (2008b) may include firmness and consistency index, which relate to the strength of the aggregated particle to particle network system. Chocolate hardness is a physical property defined in this context as the maximum force penetrating into a bar of chocolate. Increasing the lecithin concentration from 0.4% to 0.6% had no significant ($P>0.05$) effect on chocolate hardness at 30 min wet conching time (Figure 6.12).

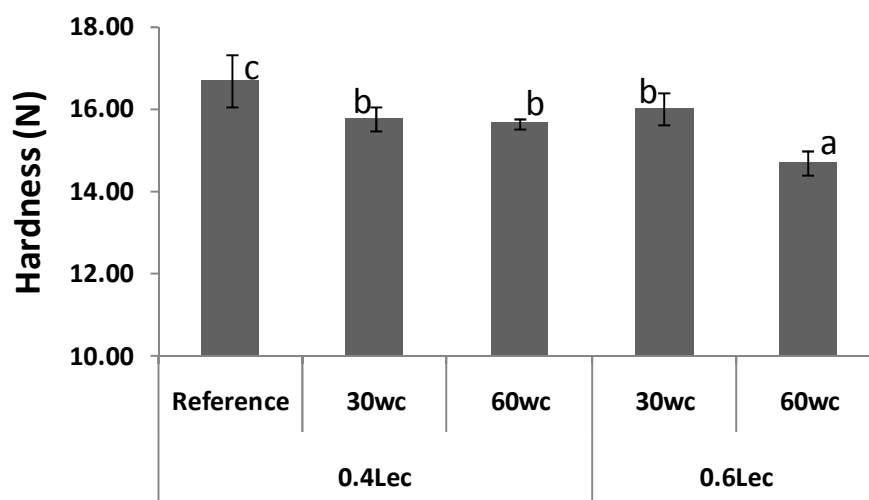


Figure 6.12 Effects of wet conching time and lecithin concentration on texture of sugar-free chocolate [samples with different letters are significantly ($P<0.05$) different]

A significant ($P < 0.05$) reduction in chocolate hardness was however observed with increase in lecithin concentration at 60 min wet conching time. Afoakwa et al. (2009) reported decrease in hardness of conventional dark chocolates with increase in lecithin concentration. The authors associated this with the amphiphilic properties of lecithin, which increases de-agglomeration of clumps and wetting, thereby inducing softening in chocolate. Increasing wet conching time from 30 min to 60 min at 0.6% lecithin concentration also resulted in a significant ($P < 0.05$) reduction in the chocolate hardness (Figure 6.12). This can be attributed to efficient coating of solid particles with cocoa butter at longer wet conching times resulting in reduced particle aggregation and consequently reduced chocolate hardness.

6.6.3.3 Moisture

Increasing lecithin content of chocolates from 0.4% to 0.6% caused significant increases in moisture content of products, a trend noted at all wet conching times (Figure 6.13). Increasing wet conching time however had no significant effect on moisture content for both 0.4% and 0.6% lecithin concentrations. The increase in moisture content with increasing lecithin concentration (0.4 to 0.6%) could be attributed to the formation of a thin film of fat on the surface of solid particles as a result of extra added lecithin which prevents water removal thereby enhancing the toleration of higher moisture levels (Lucisana et al., 2006).

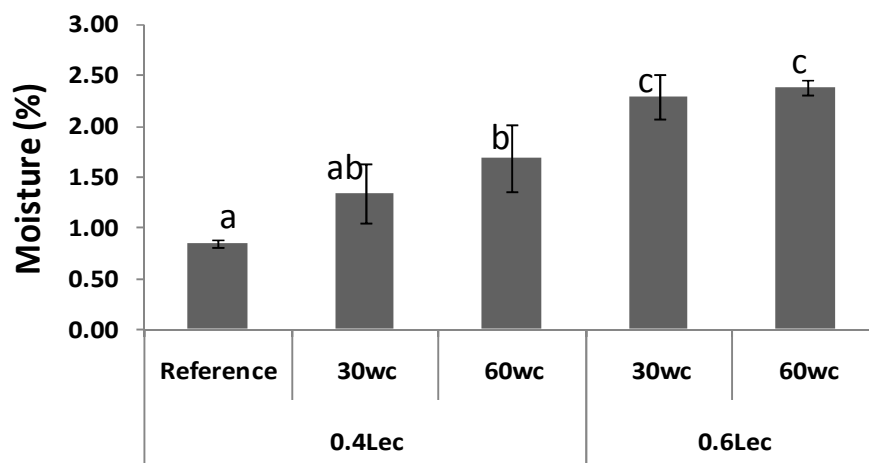


Figure 6.13 Effects of wet conching time and lecithin concentration on moisture content of sugar-free dark chocolate [samples with different letters are significantly ($P < 0.05$) different]

6.6.3.4 Colour

Variation in lecithin concentration and wet conching time resulted in similar L^* values for the chocolate samples (Figure 6.14). This was confirmed by ANOVA, which showed that both wet conching time, and lecithin concentration had no significant effect ($P>0.05$) on the colour parameter. Therefore, regardless of the amount of lecithin added or time used for wet conching, chocolates colour did not change. Chocolate composition and PSD have already been established as factors that causes changes in colour in sucrose-free dark chocolates and since the bulk composition of the chocolate (75%PD:25%IN) was not varied, the colour did not change.

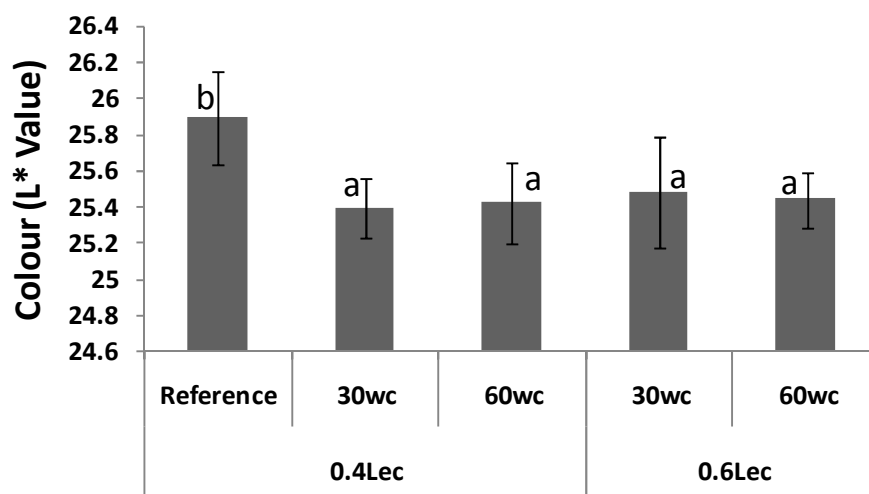


Figure 6.14 Effects of wet conching time and lecithin concentration on Colour of sugar-free dark chocolate [samples with different letters are significantly ($P<0.05$) different]

6.6.3.5 Melting properties

Overall, wet conching time and lecithin concentration had very little influence on chocolate melting properties. Increasing the lecithin concentration from 0.4% to 0.6% however resulted in a significant reduction in maximum temperature (T_{max}) at 30 min wet conching time.

Table 6.4 Effects of wet conching time and Lecithin concentration on melting properties of sugar-free dark chocolate.

Wet conching time (min)	Lecithin (%)	Onset T (°C)	Maximum T (°C)	Offset T (°C)	Energy (W/g)
30	0.4	31.80 ± 0.43 ^a	35.04 ± 0.08 ^b	37.52 ± 0.44 ^a	36.41 ± 0.55 ^a
	0.6	31.65 ± 0.29 ^a	34.39 ± 0.15 ^a	36.67 ± 0.40 ^a	37.05 ± 0.36 ^a
60	0.4	31.71 ± 0.87 ^a	34.86 ± 0.30 ^{ab}	37.52 ± 0.19 ^a	36.50 ± 0.12 ^a
	0.6	31.76 ± 0.94 ^a	34.50 ± 0.08 ^a	37.02 ± 0.49 ^a	36.83 ± 0.56 ^a
Reference	0.4	30.47 ± 0.34 ^a	35.08 ± 0.33 ^b	37.57 ± 0.15 ^a	36.78 ± 0.25 ^a

Means ± standard deviations from triplicate analysis, means within a column with different letters are significantly different ($P < 0.05$)

6.7 Conclusions

Decreasing fat content impacted negatively on the sugar-free dark chocolates by increasing Casson yield stress and Casson viscosity. Increasing inulin concentration with simultaneous reduction in polydextrose concentration also resulted in an increase in Casson viscosity, a trend noticeable at all fat contents. This was attributed to the low density of inulin compared to polydextrose, resulting in increased particle volume fraction and particle surface area for formulations with high levels of inulin. The Casson yield stress of chocolate formulations with high polydextrose concentrations (75-100%) at 30 and 33% fat contents were not significantly different from the reference samples. The colour (L^* -values) of chocolate formulations with 100% PD were also not significantly ($P > 0.05$) different from their reference samples at 30% and 33% fat content. Lecithin concentration and wet conching time also had significant ($P < 0.05$) effect on chocolate flow properties. A significant reduction in Casson viscosity was observed with increase in wet conching time at both lecithin concentrations (0.4% and 0.6%). Increasing the lecithin concentration from 0.4% to 0.6% however had no significant ($P > 0.05$) effect on the Casson yield stress at both wet conching times (30 and 60min). Thus, lecithin can be added to the sugar-free dark chocolate up to concentrations of 0.6% without any increase in the Casson yield stress.

**Chapter 7: Equivalence sweetening and bitterness sensations
evaluated by time-intensity measurements on different
stevia-rebaudioside A sweeteners and thaumatin as sucrose
replacers in sugar-free chocolate manufacture**

This chapter has been submitted to International Journal of Food Science and Technology and is currently under review

7.1 Introduction

Sweet taste predominantly produced by sugar plays an important role in food preferences. While sucrose remains the most commonly used sugar and a popular ingredient to obtain sweetness in human food preparation (Jamieson, 2008; De Baets, 2010), dietary and health demands continue to expand the market for sweeteners as alternatives to sucrose (Portmann and Kilcast, 1996; Aidoo et al., 2013). High intensity sweeteners are well known alternatives to nutritive sweeteners, providing sweetness without the calories and the other metabolic impact of nutritive sweeteners. Sucrose replacement thus serves a number of purposes - expanding food and beverage choices for those who want to control caloric, carbohydrate or sugar intake, aiding in the management of diabetes and providing sweetness when sugar is not available (De Melo et al., 2007). However, such replacements should not cause significant changes in the sensory characteristics of the product (Bolini-Cardello et al., 1999).

Recently, the launching of new products containing stevia extracts has increased, while those containing artificial sweeteners have decreased (Aidoo et al., 2013). Rebaudioside A is a high potency diterpenoid glycoside sweetener isolated and extracted from the *Stevia rebaudiana* (Bertoni) plant ("stevia"), which is commercially cultivated in Japan, Singapore, Taiwan, Malaysia, South Korea, China, Israel, India, Brazil, Australia, and Paraguay (Aidoo et al., 2013; Mosettig and Nes, 1995). It is an alternative non-caloric sweetener with functional and sensory properties superior to those of many high-potency sweeteners. Processed forms of *Stevia* can be 70 to 400 times more potent than sucrose, however, some *Stevia* products possess strong bitter persistent aftertastes making them unsuitable for use in confectionery products. Of the four major diterpenoid glycoside sweeteners present in *Stevia*, rebaudioside A has been identified as the least bitter, and with the least persistent aftertaste (Prakash et al., 2007). The bitterness is significantly due to the impurities in extracts. According to Prakash et al. (2007), it is very difficult to obtain a high purity of rebaudioside A in high recovery because rebaudioside A and the impurities have similar solubility. Differences therefore exist among sweeteners for initial and residual sweet intensities and non-sweet aftertaste (Ott et al., 1991). Rebaudioside A is available at moderate cost at $\leq 80\%$ purity and at $>80\%$ purity only at high cost. Previously reported efforts to purify rebaudioside A and stevioside require numerous repeated purification

steps. Accordingly, there is the need to check the sensorial characteristics of commercially available stevia extracts claiming to have certain levels of purity and their suitability as sucrose replacers in chocolate manufacture.

Thaumatocin is an intensely sweet-tasting protein isolated from the arils of *Thaumatococcus daniellii* Benth, a plant native to tropical West Africa (Ohta, 2011). Thaumatocin is the most characterized of the sweet proteins. Thaumatocin is 100,000 times sweeter than sugar on a molar basis and 3,000 times on a weight basis (De Vos, et al., 1985). The onset of sweetness due to thaumatocin is relatively slow with a slight liquorice aftertaste. The safety of thaumatocin has been proven for animal and humans. It does not cause tooth decay and can be used by diabetics. In 1983, the use of thaumatocin was approved by Great Britain for dietary products, drinks, pharmaceutical products and foods (excluding those intended for babies). Thaumatocin is therefore considered by consumers as a novel type of food additive with new properties (Gibbs et al., 1996).

The Time-intensity (T-I) method is a useful tool which makes it possible to compare the perception of sucrose sweetness over time with that of other sweeteners (De Melo et al., 2007; Aidoo et al., 2013). T-I data collection techniques provide much more information, addressing rate-related and duration aspects as well as intensity quantification (Lee, 1989). Thus, T-I method scaling has increased in popularity as an applied sensory evaluation method (Clark and Lawless 1994). De Melo et al. (2007) concluded that the T-I method can be a first experiment to select a potential high-intensity sweetener and its concentration to replace sucrose. Time-intensity profiles generate a series of temporal parameters for quantifying sweeteners and provide a valuable method for discriminating sweeteners on the basis of the onset (the time when sweet taste is first perceived), intensity and duration (the time until no sweet taste remains) of perception of a sensory attribute (Portmann and Kilcast, 1995). Ayaya and Lawless (1992) reported that time intensity data may better approximate conditions of consumption and be more suitable for examining the sweetness of intense sweeteners. Typically, T-I parameters as maximum intensity, time to extinct perception and area under the curve are used for examining the functionality of different sweeteners. Thus, the potency of sweeteners could be predicted from the temporal profiles of several concentrations of both, test and reference sweeteners (Calviño et al., 2000).

7.2 Research strategy

Stevia rebaudioside A and thaumatin are considered natural sweeteners and possess varying sweetening potentials and aftertastes not suitable in certain confectionery products. This research was aimed at comparing and selecting the most suitable commercially available Stevia rebaudioside A and thaumatin sweeteners with similar sensory characteristics as sucrose, for use in sugar-free chocolate production. The sweetness potencies, bitterness levels and sweetness profiles of three rebaudioside A products from different suppliers (Q, R and S) - having same level of purity (Reb A 97%) - were sensorially analysed using the time-intensity (T-I) method as a tool, with slight modifications. This was done to assess the suitability of replacing sucrose with Stevia rebaudioside A or thaumatin in the manufacture of sugar-free chocolates. Since concentration influences T-I profiles, different concentrations of the intense sweeteners were studied in order to expand the database regarding T-I properties of the commercially available sweeteners.

7.3 Materials and methods

7.3.1 Raw materials

Three Stevia – rebaudioside A (Reb A 97%) products from different suppliers were obtained and studied. For the purpose of this research, the samples were labeled Q, R and S representing Reb A (97%) from suppliers 1, 2 and 3, respectively. Sucrose was obtained from Barry Callebaut (Wieze, Belgium). Thaumatin was obtained from Samatex Thaumatin Company (Takoradi, Ghana).

7.3.2 Panel screening and training

Ten subjects were recruited among students and staff at the Faculty of Bioscience Engineering, Ghent University, who showed interest in becoming members of the sensory group to be trained. The panel constituted 6 females and 4 males. The distribution of the panelists by country is as follows (4 Belgians, 3 Vietnamese, 1 Iranian, 2 Indonesians). They were selected on basis of (i) availability throughout the study (ii) performance on basic taste recognition tasks (iii) ability to ascertain degrees of differences for sweet and bitter stimuli

at different concentrations and (iv) repeatability (Damásio and Costell 1991), as verified by triangular tests and the Wald sequential analysis (Amerine et al., 1965). Panelists were trained over 2 weeks to become acquainted with the sensory techniques used for the time-intensity assessment. Three sucrose references (0, 25, and 50% w/v) were used to normalize sweetness intensity ratings on a 9 cm scale. These sucrose standards were allotted the intensity values 0, 4.5, and 9, respectively. Two quinine solutions were used as reference for bitterness with 0.006% assigned a bitterness value of 2 and 0.03% a bitterness value of 8 (Carr et al., 1993; DuBois et al., 1991). The intensity references were determined for the attribute of sweetness and bitterness so that the extremes of the scale could be established for all the subjects. For time-intensity evaluation, practice sessions were held using 5 and 10% sucrose solutions and 5 and 10% SEV (sucrose equivalent value) sweetener solutions to give panelists an awareness of various taste intensities and degree of lingering tastes. A discussion session followed to agree on the profiles and timings for the evaluations.

7.3.3 Sample preparation

Samples were dissolved in Louise water (Louise Spring Water Co. Ltd, Brugge, Belgium) 24 h before the evaluation and stored at 4 °C overnight. Concentrations were reported on a weight/volume basis. Panelists received 15 ml aliquots of test stimuli, served at room temperature (22 °C) in 30 ml odour-free plastic cups coded with randomly selected three-digit numbers.

7.3.4 Sensory procedures

Tests were carried out in individual tasting booths in the Sensory Analysis Laboratory (SensoLab) of the Faculty of Bioscience Engineering, Ghent University and samples were evaluated under red filter lights to minimize appearance differences of solutions. Panelists were given a brief outline of the objectives of the work, but with no information on the suppliers of the sweeteners. Data collection was by printed ballot sheets with 9-cm structured linear scales corresponding to the specific times 5 s, 10 s, 20 s, 30 s, 40s, 50s and 60 s for the time intensity studies (Figure 7.1). In all, four concentrations of the rebaudioside A samples (0.14; 0.18; 0.22 and 0.26% w/v), covering its sweetness potency range (200 - 300 X sweeter than sucrose) reported in literature (Jamieson, 2008), and three concentrations of thaumatin (0.05; 0.06; and 0.07% w/v) based on preliminary experiments

in comparison with sucrose were evaluated. The panel facilitator kept a timer to inform the panelists of the times for evaluating the sweetener attributes.

7.3.4.1 Maximum sweetness intensity (*I_{max} stat*)

The evaluation of the maximum sweetness intensity for each sweetener was carried out using T-I analysis. Panelists began by tasting a sucrose reference (50% w/v) before tasting the test samples. Panelists sipped a first aliquot and swallowed. At the signal of the facilitator after 5 s, they registered a mark on the ballot sheet with the printed scale with a pen as the maximum sweetness intensity (*I_{max} stat*). The left-hand end of the scale was labelled 0 and was defined as having a sweetness equivalent to a 0% sucrose solution.

5 s	0 (None)	4.5 (Moderate)	9 (Strong)
10 s	0 (None)	4.5 (Moderate)	9 (Strong)
20 s	0 (None)	4.5 (Moderate)	9 (Strong)
30 s	0 (None)	4.5 (Moderate)	9 (Strong)
40 s	0 (None)	4.5 (Moderate)	9 (Strong)
50 s	0 (None)	4.5 (Moderate)	9 (Strong)
60 s	0 (None)	4.5 (Moderate)	9 (Strong)

Figure 7.1 Printed ballot sheet with structured line scales used for T-I analysis.

The right-hand end of the scale was labeled 9 and was represented by a sweetness equivalent to a 50% w/v sucrose solution. Evaluation of all the samples was conducted in a similar fashion. After evaluating one concentration level for all the samples, panelists took a break of 10 min to reduce the effect of fatigue. Panelists were instructed to rinse their

mouth before each session and after tasting each sample. The rinsing protocol consisted of eating a piece of cracker and then rinsing their palates with warm water to dissolve any sweetener residues. All assessments were carried out in triplicate.

7.3.4.2 Bitterness level

The evaluation of the level of bitterness of the samples was carried out by single point determination, without time measurement. Panelists began by tasting the quinine reference (0.006% w/v) before tasting the test samples. Panelists sipped a first aliquot and swallowed. They were instructed to score bitterness level at the point of maximal bitterness on the structured linear scale, by making a mark on the ballot sheet with a pen. The left-hand end of the scale was labelled 2 and was defined as having a bitterness equivalent to a 0.006% w/v quinine solution. The right-hand end of the scale was labelled 8 and was represented by a bitterness equivalent to a 0.03% w/v quinine solution. Evaluation of all the samples was conducted in a similar fashion. After evaluating one concentration level for all the samples, panelists took a break of 10 min to reduce the effect of fatigue. Panelists were instructed to rinse their mouth before each session and after tasting each sample. The rinsing protocol consisted of eating a piece of cracker and then rinsing their palates with warm water to dissolve any sweetener residues. All the assessments were carried out in triplicate.

7.3.4.3 Time-intensity analysis

Collection of T-I data included an initial measurement of intensity followed by the T-I measurements. The data collection for the time-intensity analysis was carried out on the printed 9-point structured linear scales (Fig. 7.1), each scale representing the times (5, 10, 20, 30, 40, 50 and 60 s) for evaluating the sweetness intensities of the given samples. The structured line scale is a continuous horizontal line of 9 cm in length with three vertical lines, indicating the numbers 0, 4.5 and 9. On top of the scale were words that indicate the intensities, where 0 corresponds to none (far left), 4.5 corresponds to moderate (middle) and 9 corresponds to strong (far right). An example of the structured line scale is as shown in figure 7.1. A mark to the right indicated increase in sweetness and a mark to the left indicated a decrease in sweetness. Panelists sipped a first aliquot and swallowed. At the signal of the facilitator after 5 s, panelists indicated the intensity of sweetness by registering a mark on the ballot sheet with the scale for 5 s. On hearing the second signal after 10 s, the

panelist again indicated the intensity of sweetness of the sample on the tongue on the scale for 10 s. The intensity of sweetness on the tongue was then rated over a period of 60 s at 10 s intervals. The onset time and total duration of sweetness measurement were kept constant for all samples for the purpose of comparison. Each session consisted in the evaluation of the three sweeteners at one concentration level in random order together with the reference sample (48% sucrose solution). Consequently, the whole experiment consisted of 12 sessions, during which three T-I replicates were obtained for each concentration of sweetener. At the beginning of each session panelists were presented with standard sweet solutions (0, 25 and 50% w/v sucrose) to define intensities equivalent to 0, 50 and 100% of the full scale.

7.3.5 Data analysis

Intensity ratings on the line scales were converted into numbers by measuring the distance, in centimeters, of each subject's rating from the left end of the line. Average ratings of sweetness and bitterness intensities were calculated for each sweetener at the range of concentrations. T-I curves were obtained with Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, WA, USA). Individual data points on the T-I graphs represent the average of all panelists' intensity scores. T-I parameters were defined as follows: *Imax stat*: maximum intensity obtained at the start, *Imid*: intensity after 30 s, *lend*: intensity after total duration of sweet response at 60 s. The comparative analysis consisted of an ANOVA considering the products and concentrations as factors, followed by Tukey's honestly significant (HSD) average test for each of the effects produced by the T-I parameters to check if there are differences between the samples at a 5% significance level ($p \leq 0.05$). The statistical analyses were carried out using the software Statgraphics Centurion XV (Graphics Software System, STCC, Inc., Rockville, USA).

7.4 Results and discussion

7.4.1 *Stevia rebaudioside A*

The mean intensity ratings for *I_{max stat}*, *I_{mid}* and *I_{end}* of each stevia sweetener along with standard deviations are given in Table 7.1. The time-intensity profile of the stevia sweeteners at different concentrations compared to the reference sample of 48% sucrose is shown in Figures 7.2A, 7.2B, 7.2C and 7.2D. The average ratings of bitterness for each stevia sweetener at concentrations of 0.14, 0.18, 0.22 and 0.26 % w/v are plotted in Figure 7.3.

7.4.1.1 Sweetness profile

It is widely believed that consumer acceptability of different intensive sweeteners depends on the similarity of their time profile to that of sucrose (Lawless and Heymann 1999). Accordingly, work to improve sweeteners has been directed to mimicking sucrose and concerned with only one sense-taste. Alternative sweeteners are successful if they match perfectly the taste quality of sucrose (Portmann & Kilcast, 1995; Aidoo et al. 2013). The sweeteners exerted a different sweetness intensity perception pattern compared to that of sucrose within the time frame of 60 s as shown in Figure 7.2. None of the intense sweeteners was perceived exactly as sucrose. At a concentration of 0.14% w/v the sweetness profile for sample Q looked quite different from that of samples R and S and much similar to the reference. Sample Q also seemed to be the only sweetener with consistent decrease in sweetness intensity with time unlike samples R and S whose sweetness intensity dropped quiet faster after 20 s of evaluation.

As concentration increased from 0.18 to 0.22% w/v no clear differences in sweetness profiles were observed for all three rebaudioside A samples. The sweetness intensity for all samples measured at 0.18% w/v decreased much faster between 5 to 30 s than between 30 to 60 s where there was a decrease in sweetness intensity with increase in time but at a much slower rate (Fig. 7.2B). In contrast, the decline in sweetness intensity was much faster right from the onset to the end of tasting at the concentration of 0.22% w/v. At the maximum concentration of 0.26% w/v however, a clear difference was observed between the sweetness profiles of the three sweeteners. Sweetness profiles of samples R and S showed an uneven (wave-like) sweetness decline trend with increase in time. In addition,

sample S presented the lowest intensity ratings for the entire duration of the ratings. According to Portmann and Kilcast (1996), concentration influences the degree to which sweetener differences can be perceived.

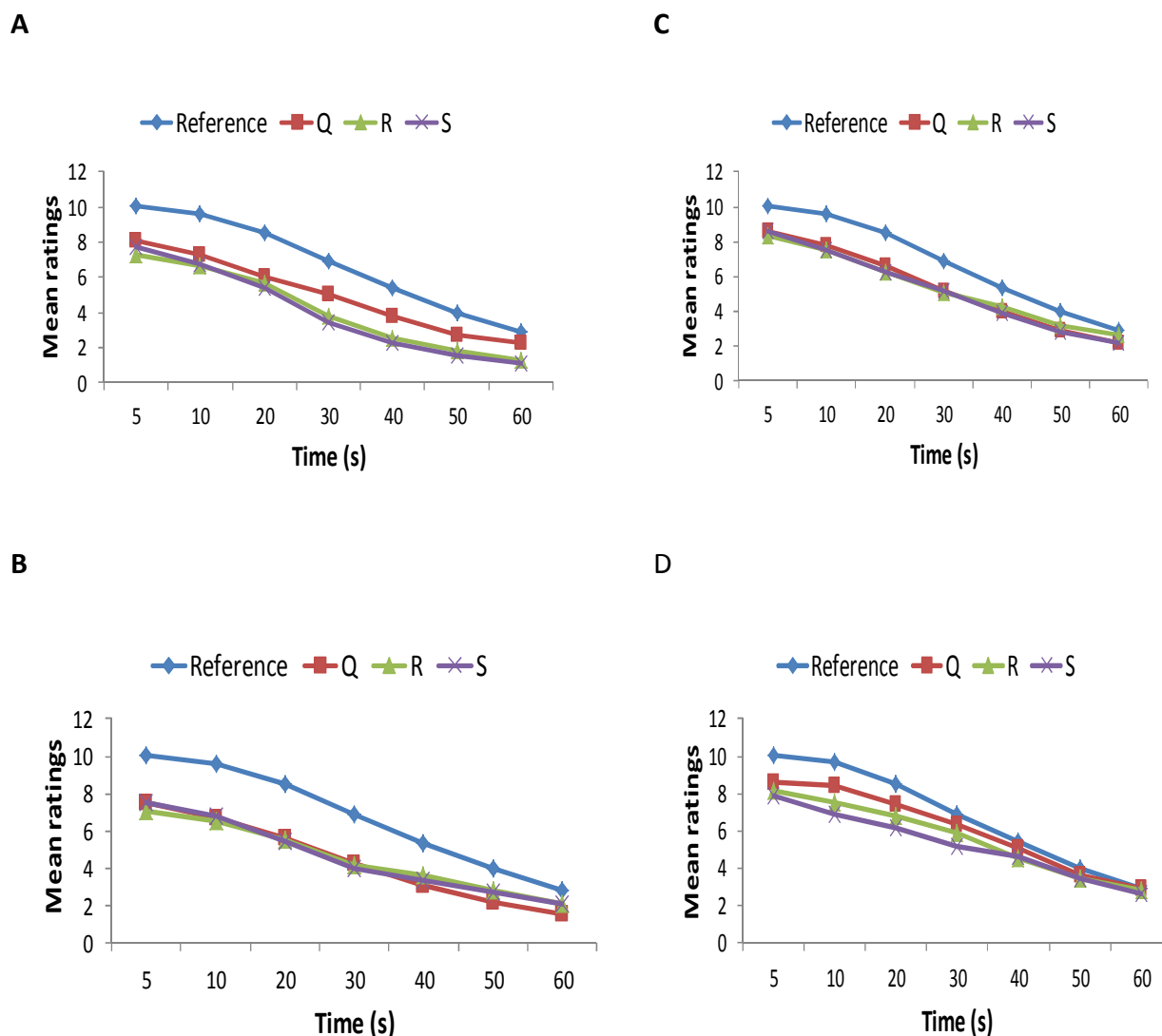


Figure 7.2 Time intensity curves for three Rebaudioside A products (Q, R and S) with same level of purity (Reb A 97%) at different concentrations. (A) 0.14% w/v (B) 0.18% w/v (C) 0.22% w/v (D) 0.26% w/v

Sweetener differences become more evident at high concentrations (Portmann and Kilcast, 1996). The inconsistencies in the sweetness decline with increase in time for sample S could have been as a result of high bitterness levels causing each panelist to evaluate the sweet taste in an individual manner. At high concentrations the bitter aftertaste of rebaudioside A could have impacted on the sweetness of the samples. Bolini-Cardello et al. (1999) reported

that panelists observed an increased bitter residual taste with increased concentration, nearly covering the sweet taste starting at concentration equisweet to 20% sucrose for stevia leaf extract. Sample Q however stood out as the sweetener with a profile most similar to the reference at concentration of 26% w/v, recording the highest intensity ratings at all times.

7.4.1.2 Bitterness levels

Both natural and synthetic sweeteners have been described as having some bitter characters (Ott et al., 1991). Rebaudioside A often has a bitter aftertaste believed to be due to impurities which can impair or even change its sweetness perception at high concentrations (Prakash et al., 2007). Differences in bitterness ratings for the rebaudioside A samples were observed at all concentrations (Fig. 7.3), indicating differences in the level of purity of the rebaudioside A. Bitterness rating also increased with increase in concentration above 0.18% for all samples (Fig 7.3). The implication of such results is that the trend for higher bitterness ratings to be given to higher concentrations was consistent over subjects (Fig. 7.3).

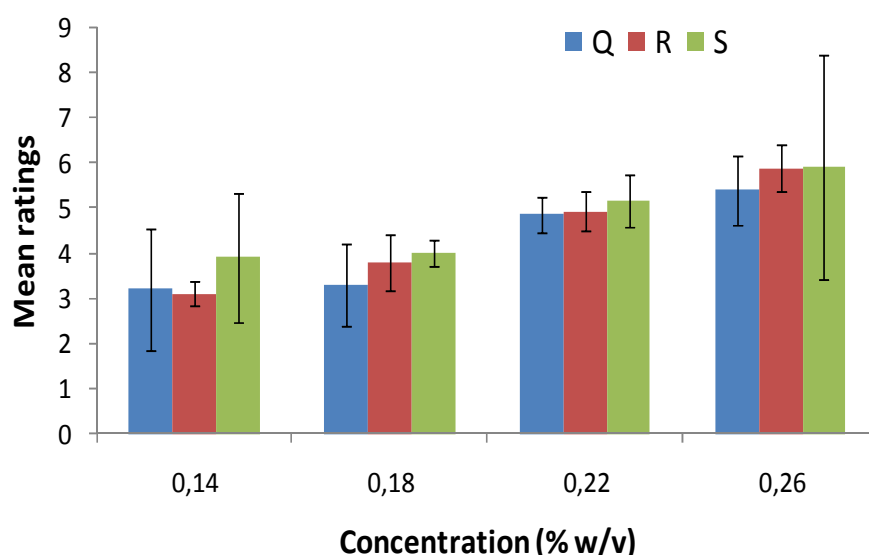


Figure 7.3 Mean bitterness ratings for three Rebaudioside A products (Q, R and S) with same level of purity (Reb A 97%) at different concentrations.

According to Schiffman et al. (1995), samples bitterness ratings increases with concentration for sweeteners that are considerably more potent than sucrose. Overall, increase in bitterness ratings from 0.14% w/v to 0.18 % w/v was by a lower margin compared to bitterness ratings from 0.18 % w/v to 0.22 % w/v and from 0.22 % w/v to 0.26 % w/v, especially for samples Q and S. Sample S recorded the highest bitterness ratings at all concentrations recording an average rating of 3.9 at 0.14 % w/v and 5.9 at 0.26 % w/v. The sample with the lowest bitterness ratings at most concentrations was sample Q, although, sample R recorded the lowest average bitterness rating of 3.1 at 0.14 % w/v. In addition, sample Q showed the least increase in bitterness ratings from 0.22 to 0.26 % w/v compared to samples R and S, indicating differences in the level of impurities present in the rebaudioside A samples which can have negative impact on their sensorial properties.

ANOVA on Time-intensity Parameters

To compare the temporal characteristics of the sweeteners, T-I parameters were subjected to ANOVA (Table 7.1). Although the standard deviations are high, the sweeteners tend to increase in maximum sweetness intensity (*Imax stat*) with increasing concentration up to 0.22 % w/v after which there was a decrease in the sweetness intensity (*Imax stat*). This observation could have been as a result of the bitter aftertaste usually associated with stevia extracts especially at higher concentrations. The other T-I parameters *Imid* and *Iend* however showed an increase in sweetness intensity with increase in concentration throughout the concentration range. Sweetener type and concentration were highly significant sources of variation for all T-I parameters. There was however no statistically significant interaction ($P>0.05$) between sweetener type and sweetener concentration for all studied T-I parameters. Sample Q recorded the highest maximum sweetness mean rating (*Imax stat*) at most concentrations but was not statistically significant from samples R and S at the 5% significance level. Multiple range test using Tukey's honestly significant difference (HSD) thus failed to detect inter-sweetener differences for *Imax stat* at the 95.0% confidence level. Increasing concentration led to a statistically significant effect on *Imax stat*.

Table 7.1 T-I parameters of rebaudioside A samples at different concentrations (**I_{max} stat**: maximum sweetness intensity at start; **I_{mid}**: sweetness intensity after 30 s; **I_{end}**: sweetness intensity after total duration)

Concentration (% w/v)	Sweetener type	T-I parameters		
		I _{max} stat (mean±SD)	I _{mid} (mean±SD)	I _{end} (mean±SD)
0.14	Q	7.56±2.08 ^a	5.02±3.24 ^{ab}	2.24±1.80 ^a
	R	7.11±2.16 ^a	3.83±2.57 ^{ab}	1.25±1.58 ^a
	S	7.54±2.00 ^a	3.42±2.76 ^a	1.14±1.29 ^a
0.18	Q	8.06±1.92 ^{ab}	4.26±2.99 ^{ab}	1.55±1.69 ^a
	R	7.25±1.62 ^a	4.17±2.95 ^{ab}	2.10±2.14 ^a
	S	7.71±1.66 ^{ab}	4.04±2.75 ^{ab}	2.08±2.08 ^a
0.22	Q	8.61±2.11 ^{ab}	5.2±3.26 ^{ab}	2.15±1.91 ^a
	R	8.31±1.74 ^{ab}	5.06±2.51 ^{ab}	2.63±2.61 ^a
	S	9.57±1.17 ^b	6.67±1.94 ^b	2.75±2.36 ^a
0.26	Q	8.6±0.88 ^{ab}	6.3±1.80 ^{ab}	2.84±2.36 ^a
	R	8.12±2.21 ^{ab}	5.92±2.96 ^{ab}	2.76±2.02 ^a
	S	7.87±2.61 ^{ab}	5.21±3.22 ^{ab}	2.63±2.67 ^a

Mean values were calculated across all parameters by averaging all panellists individual scores. Means within same column with different letters are significantly different ($P \leq 0.05$).

I_{max stat} at concentrations of 0.14, 0.18 and 0.26% w/v were not significantly significant from each other but *I_{max stat}* at 0.22 w/v was significantly significant from *I_{max stat}* at 0.14 and 0.18% w/v. All three samples Q, R and S were however not statistically significant from the reference sample for *I_{max stat}* at concentration of 0.22% w/v.

Sample Q recorded the highest *I_{mid}* at all concentrations except in the case of 0.22 % w/v (Table 7.1). Although mean intensity ratings for *I_{mid}* showed a clear dependence on sweetener type, there were no significant differences among sweeteners regarding *I_{mid}*. The significant source of variation between sweeteners as shown by ANOVA was essentially due to the reference sample. There was no significant difference between sample Q and the reference sample for *I_{mid}* at all concentrations. Samples R and S were also not significantly different from the reference sample at concentrations of 0.22% and 0.26% w/v. With respect to concentration, *I_{mid}* at 0.14 and 0.18% w/v was not significantly different from each other but significantly different from *I_{mid}* at concentrations of 0.22 and 0.26% w/v which were in turn not significantly different from each other. This indicates a sharp increase in sweetness intensity at 0.22% w/v concentration level for all sweeteners.

In contrast to *I_{max stat}* and *I_{mid}*, *I_{end}* for all sweeteners was not statistically significant from that of the reference. Sample Q recorded the highest mean for *I_{end}* but was not statistically significant from samples R and S. Although the mean rating of *I_{max stat}* for sucrose was higher than the rebaudioside A samples, there was no significant difference between the reference and the intense sweeteners after 60 s of evaluation. This suggests a lingering sweetness associated with the rebaudioside A samples compared to sucrose. Increasing concentration also led to a statistically significant effect on *I_{end}*. *I_{end}* at concentrations of 0.18, 0.22 and 0.26%, w/v was not statistically significant from each other but *I_{end}* at concentration of 0.14% w/v was statistically significant from *I_{end}* at 0.26% w/v. No clear trend was observed for the sweeteners and concentrations since after 60 s of sweetness rating, the bitterness in the samples probably increased affecting the sweetness ratings by the panelists.

7.4.2 Thaumatin

7.4.2.1 Sweetness profile

Only one thaumatin sample (96 % purity) was used for this research and the sweetness profiles of three concentrations (0.05% w/v, 0.06% w/v and 0.07% w/v) of the thaumatin sample was investigated to find equisweet concentration of thaumatin to 48% w/v sucrose. The concentration range was chosen based on preliminary experiments which showed that concentrations between 0.048 % w/v – 0.068% w/v of the thaumatin sample (96% purity) had sweetness levels closer to the reference. Above this concentration range, the sweetness level declined and panellists felt no sweetness but rather a weird taste. Unlike the stevia sweeteners, the sweetness profile of thaumatin was very different from the reference within the time frame of 60 s (Figure 7.4).

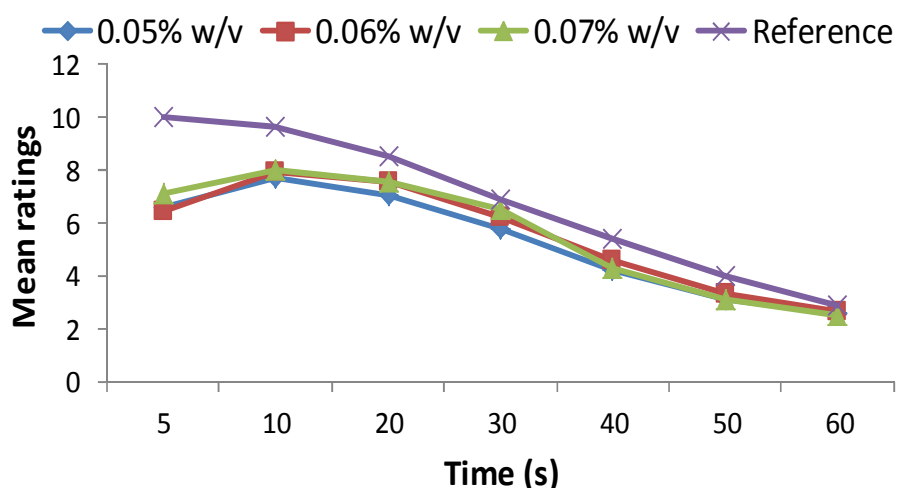


Figure 7.4 Time intensity curves for thaumatin (96% purity) at different concentrations.

The taste of thaumatin was also different from that experienced by stevia. As concentration increased from 0.05 % w/v to 0.07% w/v, no clear differences were observed. The sweetness profiles at all concentrations were not different from each other but different from the reference with a slow onset which peaked after 10 s and then declined gradually to the end after 60 s (Figure 7.4). After 50 s of evaluation, the sweetness profile of thaumatin seem to

level off in contrast to that of the reference sample which showed a continual decline between 50 s and 60 s. Thaumatin has been described as having a longer persistence in sweetness which explains the leveling-off of its sweetness profile after 50 s. Figure 5.5 compares the sweetness profiles of stevia rebaudioside A, thaumatin and sucrose.

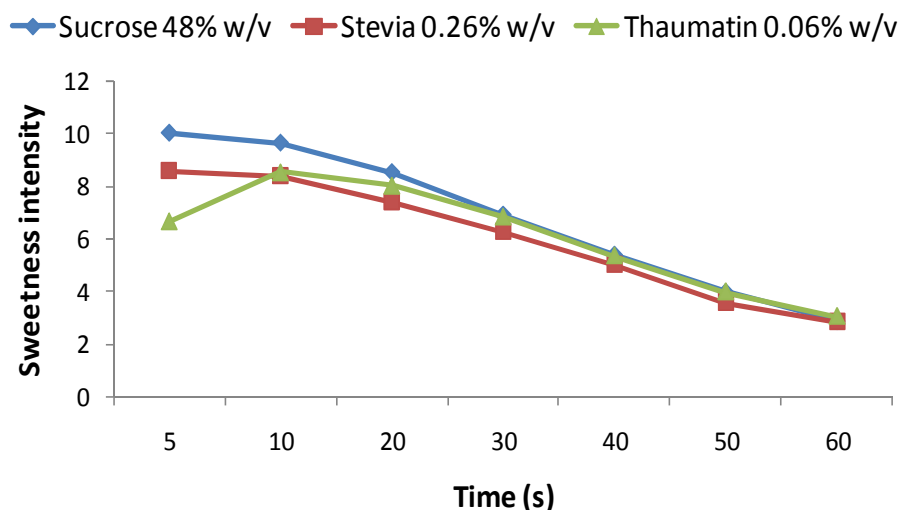


Figure 7.5 Time-intensity curves for stevia rebaudioside A, thaumatin and sucrose.

7.5 Conclusions

Quantification of time-intensity profiles represents a good way for discriminating among commercially available rebaudioside A products with same purity levels. The sweetener from supplier 1 (sample Q) was the best substitute for sucrose and presented the most suitable T-I profile for sucrose replacement. Although Sample Q was not statistically different from samples R and S for all time-intensity parameters, it recorded the highest maximum sweetness mean rating (*I_{max stat}*) at concentrations of 0.14, 0.18 and 0.26% w/v and the lowest bitterness ratings at concentrations of 0.18% w/v and higher. Concentration influenced the degree to which sweetener differences were perceived. Sweetener profile differences became more evident at high concentrations. Thus, by applying the time-intensity analysis, the rebaudioside A samples (97% purity) with concentrations between 0.24 to 0.26% w/v were considered equisweet to the reference sample of 48% sucrose solution. This is because increasing rebaudioside A concentration from 0.22 to 0.26% w/v

resulted in a marginal decrease in *I_{max stat}* and a further increase in concentration would probably increase bitter residual taste, as bitterness is a stevia extract characteristic frequently reported in literature. Differences also existed between stevia and thaumatin sweetness profiles with thaumatin exhibiting a slower sweetness onset. Thaumatin concentration of 0.06% w/v was selected as having the most suitable sweetness equisweet with the reference sample because it recorded a higher sweetness intensity compared to the other concentrations of thaumatin that was tested, even after 40 s of evaluation.

Chapter 8: Rheological, physical and sensory characteristics of sugar-free chocolates processed using inulin/polydextrose mixtures and sweetened with stevia and thaumatin extracts

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8.1 Introduction

Chocolate is a high energy product with carbohydrates, including sugar, together with fat, as the main sources of energy. It is eaten more for pleasure than for nutrition, possessing unique taste, flavor and texture. Sucrose is the most commonly used sugar in the confectionery industry, and it makes up 30-60% of chocolate depending on type (Aidoo et al., 2013). Sucrose is mainly valued for its sweetness and serves as an important source of energy, providing 394 kcal/ 100g of refined sugar. Lifestyles are becoming increasingly inactive, forcing consumers to make lower calorie food choices. Low calorie sweeteners have been available on the market for over a century as a means of providing sweet taste to foods or drinks with the benefit of little or no calories compared to sugar, thus making possible no- and low-sugar varieties of popular brands which feature in almost everyone's diet (Gibson-Moore, 2013). Specifically, interest in natural sweeteners and prebiotic compounds has dramatically increased over the past decennium.

Stevia-based sweeteners are extracted from the plant *Stevia rebaudiana* (Bertoni) and the compounds of interest are known as steviol glycosides (Boileau et al., 2012). Stevia has re-defined the category of intense sweeteners globally, because for the first time, food manufacturers have access to an effective non-calorific sweetener that is extracted from a plant and as a result has a 'natural' image (Gibson-Moore, 2013). The intensely sweet compounds are approximately 300 times as sweet as sucrose on weight basis (Geuns, 2003; Goyal and Goyal 2010), and since its approval by the European Food Safety Authority (EFSA) in 2010, consumers now have a 'natural' alternative to choose from, which may help change the perception that all low calorie sweeteners are artificial and therefore unsafe (Gibson-Moore, 2013). Stevia sweetened products are now well established in the marketplace in the UK and can be seen as table-top sweeteners and in calorie-reduced soft drinks, dairy products and some sugar-free confectionery (Boileau et al., 2012).

Thaumatococin on the other hand is an intensely sweet-tasting protein isolated from the arils of *Thaumatococcus daniellii* Benth, a plant native to tropical West Africa (Ohta, 2011). Thaumatococin is the most characterized of the sweet proteins. West African natives have used the fruit of the African rain forest shrub for centuries, to sweeten bread, tea and palm wine (Higginbotham, 1979). Commercialization came in the 1970's when Tate and Lyle began marketing thaumatococin under the trade mark, Talin, after setting up plantations in Ghana and

other West African countries (Higginbotham, 1986). Thaumatin sweetness potency and food applications are discussed under section 7.1.

Replacement of sugar with intense sweeteners such as stevia or thaumatin poses a serious challenge in chocolate confections, because sucrose fulfills both a structural and sweetening function in these products. Consumers are mostly unsure about the role and value these sweeteners have in the diet. Combination of intense sweeteners with bulking agents is needed to provide an integral solution for sugar replacement. The applicability and suitability of inulin and polydextrose as bulking agents in sugar-free chocolate manufacture have been reviewed (Aidoo et al., 2013). Sensory properties of chocolate are considered to be among the most important parameters when defining general chocolate quality. General sensory acceptance or a customer's likeability is a key factor for successful placement of a chocolate on the market as chocolate is consumed by consumers mainly for pleasure, i.e. enjoyment, and, far less, for its nutritive value.

8.2 Research strategy

Demand for use of natural sweeteners and prebiotic compounds for manufacture of sugar-free chocolates has dramatically increased over the past decennium. The main challenge of food industry is to comply with consumers' expectations that hold high standards for the foods they consume. They demand foods that taste great, are fat- and/or calorie-reduced, and they are interested in foods that provide added health benefits. Sucrose replacements by inulin and polydextrose mixtures during manufacture of sugar-free chocolates sweetened with stevia or thaumatin will be particularly interesting in view of the fact that, a good fiber-effect will be combined with a good promotion of the intestinal flora proliferation. Using cocoa liquor of high quality from Ghana, high quality sugar-free dark chocolates were produced with inulin and polydextrose as bulking agents and stevia or thaumatin as intense sweeteners. The effect of the ingredients on flow (rheological) properties, melting behaviours and physical quality characteristics was investigated. The sensory properties of the derived products were compared by consumer tests and the energy densities of the sugar-free chocolates discussed.

8.3 Materials and methods

8.3.1 Raw materials

Cocoa liquor and cocoa butter of Ghanaian origin were obtained from Cargill (Mouscron, Belgium). Sucrose (pre-broken) was supplied by Barry Callebaut (Wieze, Belgium). Soy lecithin (containing 62% acetone-insoluble matter) was supplied by Soya International (Roslyn, USA). Polydextrose (Litesse Two) was supplied by Danisco (Dordrecht, Holland). Inulin (Orafti HP) was supplied by BENE Orafti (Tienen, Belgium). Stevia rebaudioside A (Eureba Reb A97) was obtained from Bayn, Stockholm, Sweden. Thaumatin was obtained from Samatex Thaumatin Company (Takoradi, Ghana).

8.3.2 Chocolate production

Chocolate samples were prepared at UGent Cacaolab according to the formulations in Table 8.1. Chocolates were produced in batches of 4 kg per each formulation. The ingredients were mixed and refined as outlined in Section 4.3.2. The refined chocolate was dry conched in a Buhler Elk'Olino Conche (Richard Frisse GmbH Bad Salzufien, Germany) for 6h (clockwise mixing: – 1200 rpm, 60 °C, 120 min; anti-clockwise shearing: 1200 rpm, 70 °C, 120 min) with addition of 1% of extra cocoa butter to aid shearing. Lecithin and the remaining cocoa butter were added prior to wet conching at high speed (clockwise mixing: 2400rpm, 45 °C, 15 min; anti-clockwise shearing: 2400 rpm, 45 °C, 15 min) for 30 min to obtain optimum mixing and liquefaction. The resulting molten chocolate obtained was kept in sealed plastic containers at ambient temperature (20-22 °C) for further analysis. A reference chocolate sample was prepared using sucrose.

8.3.3 Tempering procedure

The tempering procedure is as outlined in section 5.3.3

8.4 Analytical methods

8.4.1 Rheological properties

The method is as outlined in Section 4.4.1

8.4.2 Moisture

The method is as outlined in Section 4.4.2

8.4.3 Hardness

The method is as outlined in Section 4.4.4

8.4.4 Colour

The method is as outlined in Section 4.4.5

8.4.5 Melting Properties

The method is as outlined in 5.4.3

8.5 Sensory analysis

Samples

The sensory tests included the two sugar-free chocolate samples, one with stevia and the other with thaumatin.

Consumer tests

The purpose of the consumer test was to evaluate the consumer preference for chocolates with total replacement of sucrose with inulin, polydextrose and stevia and chocolate with total replacement of sucrose with inulin, polydextrose and thaumatin. Thus, a comparative study of the two sugar-free chocolates sweetened with stevia or thaumatin was conducted. A consumer panel was randomly selected (n=70) from students and staff at University of Ghana. The panelists evaluated the two chocolate samples simultaneously. The samples were served in a random order in white plates. Each sample was identified with a different three-digit code. Approximately 4-5g of chocolate samples was served to the panelists, who cleansed their palates with water and crackers between assessments. Responses were recorded using a hedonic scale where untrained panelists scored from 1 to 9 for overall acceptability. A 7-point scale was used to assess the overall appearance and purchasing intent of the panelists for the chocolates. Five sensory attributes namely appearance,

sweetness, colour, hardness and stickiness were evaluated on a 1 to 5 score non-structural linear scale with grade 1 and 5 defined as too weakly or too strong expressed sensory attribute and grade 3 defined as an optimal expressed sensory attribute. The number of participants who participated in any given session was 7. Consumers' decisions were based solely on the sensory characteristics of the chocolates, since product information and formulation were not provided.

8.6 Data analysis

Data was analyzed using Statgraphics Centurion XV (Graphics Software System, STCC, Inc., Rockville, USA). One-way analysis of variance (ANOVA), and multiple comparison tests were used to determine effects of chocolate type on physico-chemical properties. Tukey's honestly significant multiple comparisons (95% significance level) determined differences between factor levels. Data entry and analysis for the sensory investigations was completed using SPSS version 16.0. Descriptive statistics were used to calculate means, standard deviations and frequencies. The t-test was applied for baseline comparisons between groups. Plotting of graphs was done using Excel spreadsheets (Microsoft, Redmont, USA).

8.7 Results and discussion

Table 8.1 presents the formulations used in chocolate formulation. Table 8.2 presents the data for moisture, hardness and melting properties of the developed sugar-free dark chocolates. The experimental results obtained for all responses were statistically evaluated and significance fixed at the 5% confidence level.

8.7.1 Rheological properties

Chocolate is processed in the molten state and as such, its rheological properties are of direct significance to manufacturing and product quality (Taylor et al., 2009). The Casson model has often been successfully applied to analyze the rheological properties of chocolates (Keogh et al., 2003; Briggs and Wang, 2004; Afoakwa, 2010; Aidoo et al., 2014a,b).

Table 8.1 Mixed composition used in sugar-free and reference dark chocolates manufacture

Treatment	Ingredients (%)							
	Cocoa liquor	Cocoa butter	Sucrose	Stevia	Thaumatococcus	Polydextrose	Inulin	Lecithin
Reference	40	11.6	48	-	-	-	-	0.4
Stevia	40	11.6	-	0.24	-	36	12	0.4
Thaumatococcus	40	11.6	-	-	0.06	36	12	0.4

Casson viscosity relates to pumping characteristics, filling of rough surfaces, coating properties and sensory character of body (Seguine, 1988). Casson viscosity reference values between 2.1 and 3.9 Pa.s has been reported by Aeschlimann & Beckett (2000) for dark chocolates. All chocolate formulations were within the range reported. This confirms earlier studies which reported that sugar-free chocolate formulations with inulin concentrations $\leq 25\%$ had viscosity values within the acceptable range (2.1 and 3.9 Pa.s) (Aidoo et al., 2014a). Combination of inulin and polydextrose in the ratio of 25:75 together with stevia or thaumatococcus as natural intense sweeteners can thus produce chocolates that can be employed for enrobing or coating as its sucrose counterpart. Although the Casson viscosity of the sugar-free chocolates were significantly ($P < 0.05$) higher than the reference chocolate (Fig. 8.1), sweetener type had no significant ($P > 0.05$) effect on the Casson viscosity. The Casson viscosities of the two sugar-free chocolates were thus not significantly different from each other since they all had same inulin/polydextrose bulk mixtures. Attaining similar flow properties in sugar-free chocolates as that of conventional chocolates is key to final product quality.

This therefore calls for more research to be done on optimization of the flow properties of sugar-free chocolates containing these ingredients. Shah et al. (2010) reported a higher plastic viscosity for chocolates with inulin HP (average DP ≥ 23 , long chain inulin) compared to control sample which confirms our findings because the source of inulin for our work is inulin HP which is the more purified form of inulin with a smooth, creamy mouthfeel. The

long chain lengths of Inulin HP according to Niness (1999), reduces its solubility and results in the formation of “inulin microcrystals” when mixed with water or milk, which are not discretely perceptible.

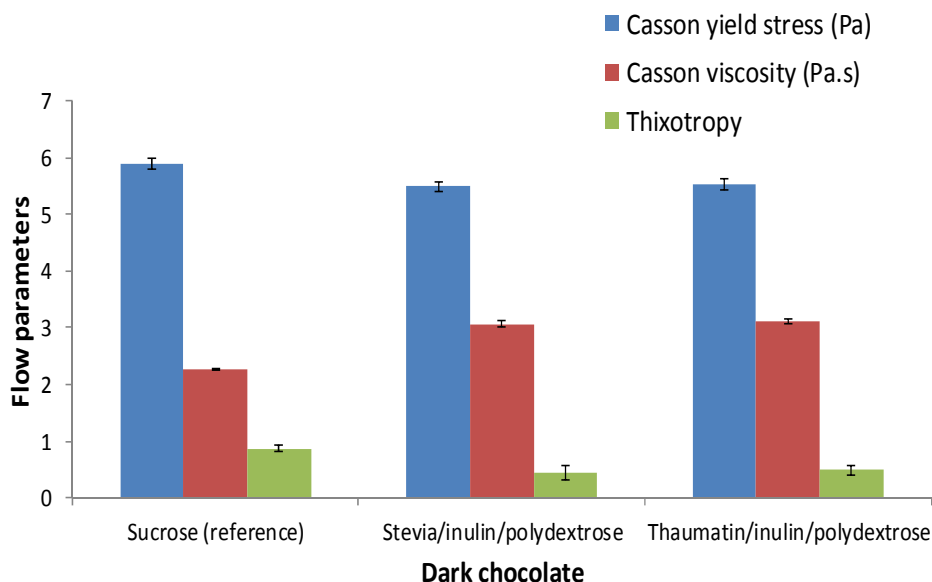


Figure 8.1 Effect of inulin/polydextrose mixture and stevia/thaumatins extracts on flow properties of sugar-free dark chocolate.

Casson yield stress is the stress required to make chocolate begin to flow. It represents the low shear-rate properties of chocolate and is affected by particle–particle interaction, the amount and specific surface area of the particles, emulsifiers, and moisture (Servais et al., 2004; Afoakwa et al., 2007; Aidoo et al., 2014a,b). The Casson yield numbers for all formulations including the reference sample (Fig. 6.1) were within the range reported for dark chocolate, i.e. 4–32 Pa (Aeschlimann & Beckett, 2000). The Casson yield value was however significantly ($P < 0.05$) higher for the reference sample (5.89 Pa) compared to the sugar-free chocolates with stevia and thaumatin, recording averages of 5.49 Pa and 5.53 Pa, respectively.

The reference sample made with sucrose also recorded a higher thixotropy than the sugar-free chocolates (Fig. 8.1). Thixotropy is exhibited in chocolates when apparent viscosity or shear stress decreases with time of shear at a constant rate, and relates to degree of

conching as well-conched chocolate should not be thixotropic (Chhabra, 2007; Beckett, 2000; Aidoo et al., 2014b). In this work, difference between yield stresses measured at a shear of 5 s^{-1} during ramp up and down in shear was used to represent thixotropy. Thixotropy value lower than 1 is a good indicator of a well conched chocolate (Afoakwa et al., 2007, Aidoo et al., 2014b). All samples showed thixotropy values below 1 (reference point) with chocolates made with stevia and thaumatin recording thixotropy of 0.44 and 0.48, respectively. ANOVA showed that, thixotropy for the two sugar-free chocolates were not significantly different from each other but significantly lower than the reference.

8.7.2 Moisture

Moisture content is an important factor in chocolate as it is closely related to the textural properties (Aidoo et al., 2014a). Overall, moisture contents of the sugar-free chocolates were higher than the reference sample but within acceptable limits of less than 1% (Table 8.2). Polydextrose and inulin are hygroscopic ingredients and as such this was expected. The right combinations of these ingredients can thus be employed to reduce the moisture contents of sugar-free chocolates to acceptable levels. Farzanmehr and Abbasi (2009) also reported higher moisture content for chocolate formulations containing sugar substitutes. The authors stated that inulin due to its low hygroscopicity did only influence the moisture content at very high levels (Farzanmehr and Abbasi, 2009). Shourideh et al. (2012) reported increase in moisture content with increase in inulin concentrations in their dark chocolate formulations containing different mixtures of D-tagatose and inulin. The authors attributed this to hydrophilic groups present in inulin which causes increase and preservation of moisture in samples with high content of inulin (Shourideh et al., 2012).

In our sugar-free chocolate formulations, only 25% of inulin was used and as such, inulin's less influence on the moisture content. This explains why some sugar substitutes are popular for replacing sugar in chocolates. The use of inulin according to Franck (2002), can allow for the development of low-fat foods without compromising the texture and mouthfeel because of their water-binding characteristics. The reference sample made with sucrose which is not as hygroscopic as the sugar substitutes recorded an average of 0.35% moisture content

which was significantly ($P < 0.05$) lower than the moisture contents of the sugar-free chocolates (Table 8.2).

8.7.3 Hardness

The effect of inulin and polydextrose mixtures on sugar-free chocolates has been studied by Aidoo et al. (2014a). The authors reported that high levels of the sugar substitutes lead to a hardening effect on chocolates with formulations containing equal concentrations of both sugar substitutes being the softest. In general, the sugar-free chocolates were softer than the reference chocolate (Table 8.2). ANOVA showed a significant effect of chocolate type on the hardness with the reference, stevia and thaumatin chocolates recording averages of 14.21 N, 13.76 N and 13.84 N respectively (Table 8.2). Farzanmehr and Abbasi (2009) reported chocolate formulation with ratios of 50:25:25% for inulin, polydextrose and maltodextrin, respectively, as the hardest indicating the dominant effect of inulin. Earlier studies reported that total sucrose substitution with inulin in chocolate resulted in the hardest chocolate (Aidoo et al., 2014a). Thus, the combination of polydextrose and inulin in the ratio of 75:25 provides an integral solution to the textural property of the sugar free chocolates. According to Shourideh et al. (2012), inulin absorbs moisture and this causes the hardness of chocolates. Sucrose replacement with inulin and polydextrose as bulking agents in the study of Shah et al. (2010), however, had no substantial effect on chocolate hardness except for chocolates made with inulin HPX.

8.7.4 Colour

Colour is one of the key attribute for consumer acceptance. Many visual attributes can be used to describe the appearance of chocolate which includes gloss, shape, surface smoothness or roughness, haze, translucency and colour (Briones et al., 2006; Aidoo et al., 2014a). Colour changes in chocolate are often due to the difference in composition and processing parameters during production. Generally, replacing sucrose with inulin and polydextrose as bulking agents and stevia or thaumatin as sweeteners resulted in darker chocolates compared to the reference (Fig. 8.2). The L^* values were significantly lower for

the sugar-free chocolates, with the reference sample, stevia and thaumatin containing chocolates recording average values of 26.85, 25.44 and 25.30 respectively. Lower values for L^* (lightness) indicate a darker appearance. This is a well reported phenomenon, as the addition of polysaccharides accelerates caramelization and Maillard reaction and therefore speeds up the formation of chocolate colour (Aidoo et al., 2014a).

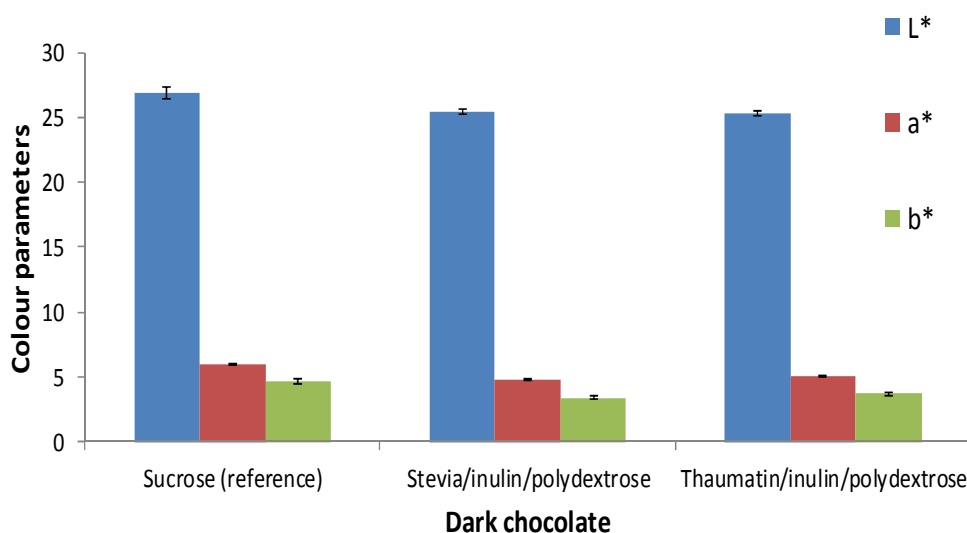


Figure 8.2 Effect of inulin/polydextrose mixtures and stevia/thaumatin extracts on colour of sugar-free dark chocolate.

Earlier studies also reported that, regardless of the levels of the sugar substitutes used, replacing sucrose with inulin and polydextrose results in darker chocolates (Aidoo et al., 2014a). Shourideh et al. (2012) reported darker chocolates for dark chocolate formulations containing 100% inulin. Inulin absorbs moisture, light scattering and lightness decreases, making the chocolate look darker (Shourideh et al. (2012). Bolenz et al. (2006) reported that chocolate samples with 20% inulin was the most brown, and had the lowest L^* (lightness) value among other texturizing agents in milk chocolate. A dark colour is usually attributed by consumers to dark chocolates hence the darker sugar-free chocolates can be considered acceptable. A decrease in the other colour parameters a^* (green to red) and b^* (blue to yellow) was also observed for the sugar-free chocolates (Fig. 8.2). This trend has also been reported by Shourideh et al. (2012). ANOVA showed all three samples being significantly

different from each other for the a^* (green to red) and b^* (blue to yellow) values indicating effect of sweetener type (stevia, thaumatin and sucrose) on the colour parameters.

8.7.5 Melting properties

The Onset temperature (T_{onset}), Peak temperature (T_{peak}), peak width at half height (T_{width}) and enthalpy of melting (ΔH_{melt}) were automatically calculated after integrating the melting peaks using TA Data analysis software (TA Instruments, New Castle, USA). Figure 8.3 shows the DSC thermograms used for evaluating the melting properties.

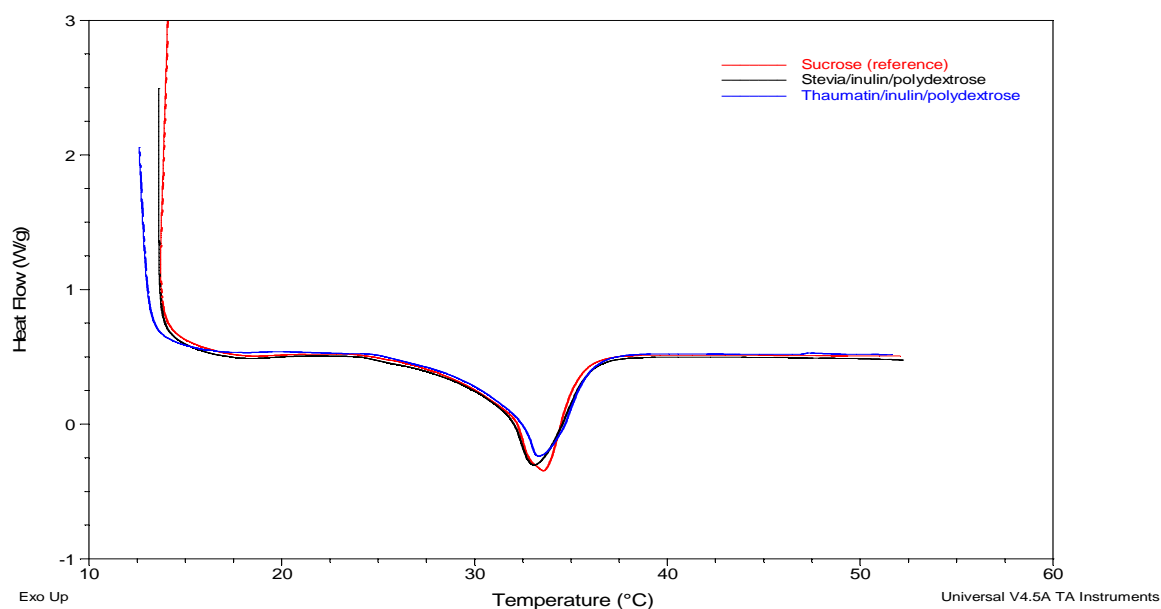


Figure 8.3 Effect of inulin/polydextrose mixture and stevia/thaumatin extracts on melting profiles of sugar-free dark chocolate.

Peak onset corresponds to the temperature at which a specific crystal form starts to melt; peak maximum, that at which melting rate is greatest; end of melting, completion of liquefaction; and peak width at half height, an indication of how long it took (duration) a particular crystal form to melt. All these information are related to crystal type (McFarlane, 1999). All the samples exhibited similar distinct single endothermic transitions between 15

°C and 55 °C (Fig 8.3). The heat capacities c_p gradually and consistently increased to onset temperature (T-onset), and then progressively increased more rapidly until peak temperature (T-peak), after which it decreased to the end temperature indicating the chocolate was completely melted (Fig. 8.3). Data from the DSC (Table 8.2) showed that sucrose substitution by the sugar replacers produced changes in crystallinity and melting properties, observed in the differences in the key DSC parameters. The onset temperature was slightly higher for the reference sample than for the sugar-free chocolates indicating a slight delay in start of melt for the reference. The enthalpy of melt was also higher for the reference than the sugar-free chocolates with the reference chocolate recording an average of 40.69 W/g. Comparing the peak width at half height for all chocolates, it was observed that, it took a slightly longer time for the sugar-free chocolates to melt with stevia and thaumatin chocolates recording average values of 3.85 °C and 3.58 °C respectively. ANOVA showed no significant differences between all samples for onset temperature at the 95% confidence level. T-peak for thaumatin chocolate was significantly different from the reference but not significantly different from stevia chocolate. A similar trend was observed for the ΔH_{melt} . There was however no significant differences in T-width for all three chocolates at the 95% confidence level.

Table 8.2 Effect of sugar substitutes on physicochemical properties of dark chocolate

Chocolate type	Physicochemical properties		Melting properties			
	Moisture (%)	Hardness (N)	Onset	Peak Max	Width	Area
			(°C)	(°C)	(°C)	(W/g)
Reference	0.35±0.02 ^a	14.21±0.07 ^a	30.60 ±1.94 ^a	33.23 ±0.78 ^a	3.47 ±0.71 ^a	40.69 ±1.17 ^a
Stevia	0.61±0.01 ^b	13.76±0.12 ^b	30.47 ±1.42 ^a	33.81 ±0.54 ^{ab}	3.85 ±0.66 ^a	38.7 ±1.96 ^{ab}
Thaumatrin	0.63±0.02 ^b	13.84±0.08 ^b	30.23 ±1.04 ^a	34.11 ±0.19 ^b	3.58± 0.58 ^a	38.03 ±1.82 ^b

Means ± standard deviations from triplicate analysis, means within same column with different letters are significantly different ($P \leq 0.05$).

The above trends can be attributed to a stronger inter-particle strength for the sucrose crystals, inhibiting the release of fat and thereby causing more energy to be used to melt the fat. It can also be associated with the microstructural behaviour of the particles of the bulk

ingredients. Earlier studies reported that chocolate formulations which contain 100% polydextrose show large crystals with dense smaller particles in between the larger crystals and minimal inter-particle spaces in comparison to formulations containing 100% inulin which revealed large crystals with more void spaces between the crystals indicating limited particle - particle interaction strength (Aidoo et al., 2014a). A combination of these ingredients will result in chocolates having large crystals with the dense smaller particles of polydextrose filling in the void spaces in the crystal network structure of chocolate formulations with inulin. The end result is chocolate with high solids packing intensity accounting for the low onset values and high peak width at half height for the sugar-free chocolates with 75:25% polydextrose: inulin ratios. The high solids packing of the sugar-free chocolates also results in a decrease in the total surface area available for fat to coat the sugar crystals hence decreasing the amount of energy needed to complete melting. Practically, although the sugar-free chocolates may begin to melt quickly than the conventional dark chocolate, it might take a longer time for all the sugar-free chocolates to melt than for the reference sample to completely melt. This knowledge is important as it provides information on likely oral melting behavior with an impact on temporal components of flavor release and also oral epithelial sensation.

8.7.6 Consumer tests

Consumer liking is the key to placing a product successfully on the market. This is especially true for chocolate, which is eaten more for pleasure than for nutrition. Sensory evaluation gives a realistic opinion about the likes and dislikes of a particular flavor or product (Hariom Shyamala et al., 2006). In consumer sensory analysis the investigator is interested in whether the consumer likes the product, prefers it to another product, or finds the product acceptable based on its sensory characteristics (Lawless & Heymann, 1998). A comparative study of the two sugar-free chocolates sweetened with stevia or thaumatin was conducted. The profiles of the panelists in our study were obtained: 62% were female and 82% were between 20 and 25 years of age. Seventy consumers evaluated the chocolate samples for liking of appearance, sweetness, hardness, colour, stickiness and overall liking. For many attributes, there was no significant ($p > 0.05$) differences between chocolates made with

stevia and chocolates made with thaumatin based on t-test statistics for equality of means (Table 8.3). The spider chart in Figure 6.4 clearly shows differences in chocolate attributes between the two chocolate samples. Average ratings for colour and texture were approximately 3.47 and 3.03 respectively, indicating that most participants rated colour and texture as “just about right” for both chocolate samples.

Table 8.3 T-test for equality of means for quality attributes of stevia and thaumatin chocolate

Attributes	Chocolates (mean scores)		t-test for Equality of Means P-value
	C1	C2	
Colour	3.50	3.43	0.495
Sweetness	1.93	1.53	0.001
Texture	2.99	3.06	0.503
Stickiness	2.43	2.27	0.274
Overall appearance	5.43	5.19	0.308
Purchasing intent	3.94	3.57	0.276
Overall acceptance	5.43	5.19	0.164

*Differences significant at $p < 0.05$ (C1=chocolate with stevia; C2=chocolate with thaumatin)

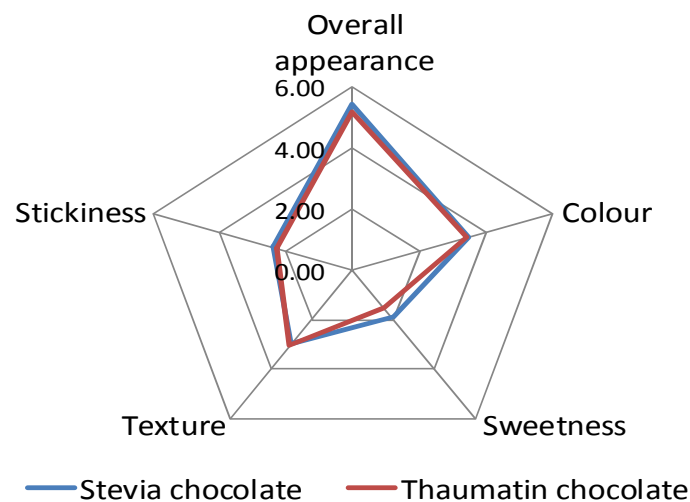


Figure 8.4 Chocolate quality attributes per chocolate type

Both chocolate samples were also rated as not too sticky with stevia and thaumatin chocolates recording averages of 2.43 and 2.27 respectively. Also noteworthy is the sweetness perception of stevia chocolate when compared to thaumatin chocolate. Although

average sweetness rating for the stevia chocolate was 1.93 indicating a sweetness intensity of “too low”, a significant difference ($P \leq 0.05$) existed between both chocolates (Table 8.3, Figure 8.4). Chocolate sweetened with stevia was rated much sweeter and better than chocolates sweetened with thaumatin. Consumers were also asked to indicate their overall liking and purchasing intent for the chocolate samples. The results are graphically represented in a spider chart in Figure 8.5.

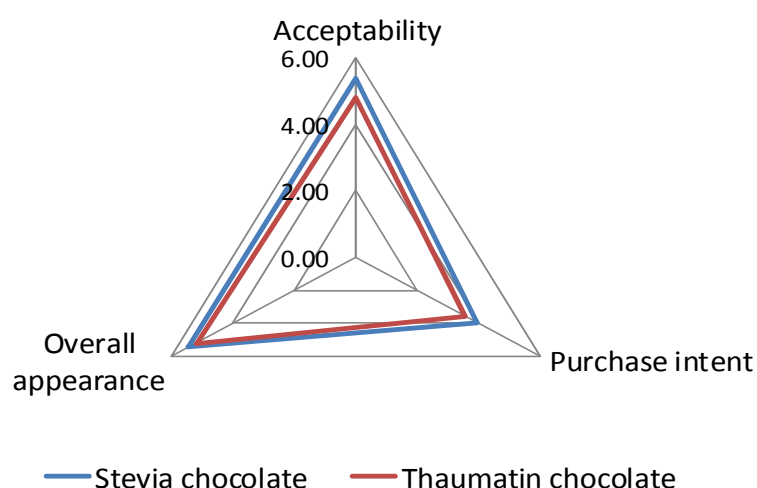


Figure 8.5 Acceptability ratings for developed sugar-free dark chocolates

In general the stevia chocolate was evaluated better than the thaumatin chocolate in terms of acceptability and purchasing intent. Results of the test on overall liking for the chocolates showed that stevia chocolate was well accepted than the thaumatin chocolate. A similar trend was observed for the test on consumer purchasing intent for the chocolates. As presented in Figure 8.6, most participants would rather buy chocolate with stevia more than the one with thaumatin and this correlated well with the overall liking of the chocolate samples. 45% of participants indicated their purchasing intent for the stevia chocolate as “probably would buy”, “would buy” or “definitely would buy” as compared to 40% of participants for thaumatin chocolate. In total, 19% were neutral as to whether they will buy either of the two chocolates by choosing the option “may or may not buy”. Sensory properties according to Dos et al. (2005) are some of the most important factors on consumer liking and preference. Luckow and Delagunty (2004) reported that consumers

would not be interested in consuming a functional beverage if the ingredients caused noticeable off-flavors that consumers found unpleasant despite the added health advantages.

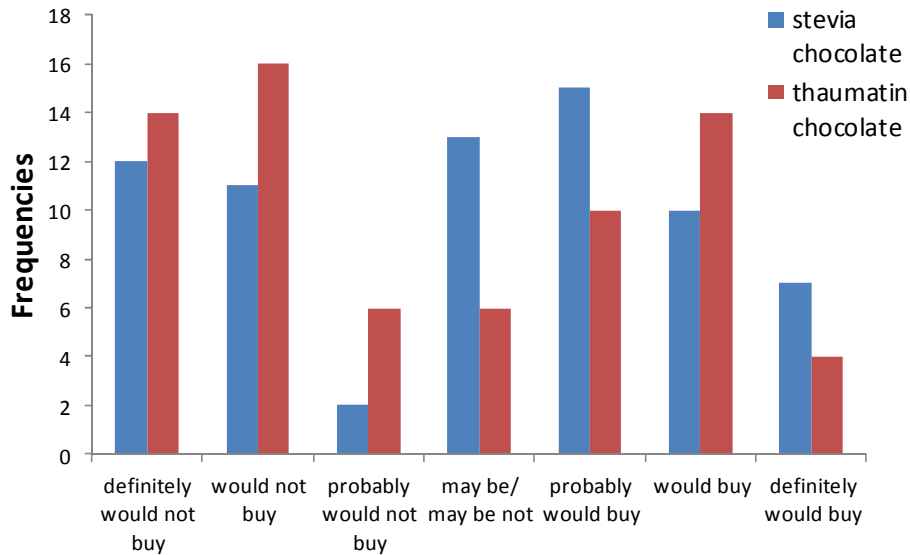


Figure 8.6 Graph showing distribution of consumer purchasing intent for sugar-free dark chocolates

Participants were asked to indicate which of the two products they preferred most based on all studied attributes. 80% of the participants indicated stevia chocolate as the most preferred chocolate. Better taste and less bitter aftertaste was the greatest motivation for choosing stevia chocolate as the most preferred chocolate. In contrast, less perceived sweetness was mentioned as the greatest deficiency of the chocolates with thaumatin. Most participants indicated that, even though both chocolates had bitter aftertastes, the stevia chocolate tasted better. The sweetness level of the stevia chocolate could however be increased a little bit for it to pass as a well acceptable sugar-free dark chocolate. Understanding what sensory properties drive consumer liking is critical for maximum market share (Thompson et al., 2004). It is very important therefore to determine factors affecting product attributes, acceptance and preference especially for foods and drinks.

Energy density of sugar-free dark chocolates

The goal of this study was to reduce the energy density of conventional dark chocolate by replacing the high caloric ingredient sucrose with lower alternatives namely polydextrose and inulin. Increasing public health demand for low-calorie, reduced sugar products has been on the rise as health problems such as diabetes, obesity and dental carries are increasing at alarming rate. The energy values of the chocolate ingredients are presented in Table 8.4. The caloric value of both polydextrose and inulin is 2kcal/g, in accordance to report by FAO on energy of fibre, through analytical methods and conversion factors that indicated that only 70 percent of fibres are assumed to be fermentable in the intestine (Directive 2008/100/EC). Sucrose provides 4 kcal/g energy and thaumatin which is a protein has 4 kcal/g. Cocoa butter is 100% fat and therefore has a caloric value of 9 kcal/g. Caloric value for cocoa liquor was calculated based on its fat content and calories from the macro composition of standard cocoa powder. The caloric content of the sugar-free dark chocolate and sucrose chocolate were therefore calculated to be 425 kcal/ 100g and 522 kcal/ 100g, respectively. Thus, total replacement of sucrose with polydextrose and inulin as bulking agents and stevia or thaumatin as intense sweeteners resulted in 19 % reduction in calories.

Table 8.4 Energy values of chocolate ingredients

INGREDIENTS	% (w/w)	Kcal/g
Sucrose	48	4
Stevia	0.24	0
Thaumatococcus	0.06	4
Polydextrose	36	2
Inulin	12	2
Cocoa liquor	40	5.6
Cocoa butter	11.6	9.0
Lecithin	0.4	3.6 ^b

^bStandard used by Nestle Product Technology Centre York, UK.

8.8 Conclusions

The substitution of sucrose with inulin and polydextrose in sugar-free dark chocolates affect product quality. Generally, the sugar-free chocolates displayed satisfactorily similar flow (rheological), melting, colour and mechanical properties to the reference. Sucrose replacement with the inulin/polydextrose and stevia/thaumatococcus extracts resulted in

significantly higher Casson viscosity. There were however no significant differences in the Casson yield value, and texture of the sugar-free dark chocolates and the reference chocolate. Chocolates containing the sugar substitutes recorded lower onset temperatures and higher peak widths than the reference sample. Consumer tests showed that a perceptible sensory difference exists between stevia and thaumatin chocolate particular for sweetness. Results from this study showed that consumers prefer the taste of chocolate with stevia to chocolate with thaumatin. This result can only be attributed to the sensory attributes associated with the alternative sweeteners, since no information was provided to influence preference. The findings of the study also indicated bitterness and bitter aftertaste as drivers for disliking the products. In general, the acceptability of chocolate with stevia sweetener was good. The caloric content was reduced by 19%. Thus, sugar-free dark chocolate with no added sugar and a source of fibre has successfully been produced using high quality Ghanaian cocoa. However, the sugar-free chocolates cannot be labeled as energy-reduced in accordance with EU regulations on nutrition and health claims which requires atleast 30% energy reduction in a food before it can be labelled as energy-reduced. The findings of this study could be applied by the chocolate industry to develop and reformulate the recipes of diabetic and/or reduced calorie chocolates to better meet consumer requirements.

Chapter 9: General conclusions and recommendations for further studies

The consumer today is health conscious and demands foods which are tasty as well as low in calories, with additional health benefits. Specifically, interest in natural high potency (intense) sweeteners and prebiotic compounds have dramatically increased in recent years. Sucrose is utilized up to 30-60% in chocolate and this confers multiple functional properties on chocolate including sweetness, bulkiness and mouthfeel (texture). Sugar's bad press coupled with the perceived naturalness halo of plant-derived intense sweeteners means the future is bright for the likes of stevia and thaumatin. Also, Inulin and polydextrose have many interesting functional attributes that meet the needs of the food industry for healthy foods. Inulin has prebiotic properties and both bulking agents are sources of dietary fibres. This work investigated the functionality of inulin and polydextrose as sucrose replacers in sugar-free dark chocolates sweetened with stevia or thaumatin.

Our findings showed that, increasing inulin content in the sugar-free dark chocolate resulted in increasing chocolate viscosity and decreasing yield stress. This was related to the particle size distribution and density of the bulking agents. Microstructural examination revealed that inulin, which had a lower density tend to have more solids per volume and increased particle volume fraction and solid's surface area, resulting in a higher particle collision and aggregation thereby limiting chocolate flow. This effect can be balanced by varying the concentrations of inulin and polydextrose mixtures or the method of addition of the bulk ingredients. All samples were refined using equal refiner settings, nevertheless particle sizes differed and tended to increase with increasing inulin content. This could be caused by slightly different breaking behaviour of the bulking agents. Thus in a technical application it would be necessary to adjust refiner settings when using inulin and polydextrose as bulking agents in sugar-free dark chocolate manufacture.

A blend of 25% inulin and 75% polydextrose had entirely positive effects on the flow, textural, melting and sensory properties of the sugar-free dark chocolates sweetened with stevia. Although the viscosity of the chocolate was high, this can be reduced by increasing the wet conching time and/or increasing the lecithin concentration from 0.4% to 0.6% without any significant changes in the yield stress. Increase in yield stress at lecithin concentrations above 0.5% have been widely reported (Chevalley, 1999; Rector, 2000; Schantz & Rohm, 2005) and is linked to the formation of bilayers around the solid particles or to the formation of lecithin micelles, both reducing the effectiveness of the emulsifier

(Beckett, 2009). Our findings however showed that lecithin concentration of 0.6% can be applied in the sugar-free dark chocolate without significant changes to the yield stress. Sugar-free dark chocolate sweetened with thaumatin received poor ratings from consumer panel and thaumatin may have to be combined with other intense sweeteners to enhance its sweetness in sugar-free dark chocolates. In the end, calorie reduction of 19% was achieved, which is below the 30% threshold to make a 'calorie reduced' claim in the EU. As such, reducing the fat content may have to be considered, which would be economically beneficial to producers. However, our findings showed that processing below 30% fat had negative effects on the tempering and moulding processes. A much simpler approach to consider would be to reduce the portion size (100g) per serving of the sugar-free dark chocolate. A survey by Mintel found that 23% of consumers believed mini-sized chocolate bars were a good way to control their chocolate consumption. Inulin and polydextrose are still highly priced than sucrose making the overall product cost higher for the sugar-free dark chocolate than the conventional dark chocolate. The health benefits of inulin and polydextrose however, cannot be overemphasized. Not only the dietary fibre properties of the bulking agents are important but also the prebiotic properties of inulin, resulting in better health and reduction in the risk of many diseases.

The process and product conditions of sugar-free dark chocolates with inulin/polydextrose mixtures as bulking agents and stevia or thaumatin as intense sweeteners have been optimized. With increasing demands for dark chocolate products in global markets and demand for innovation in chocolate and confectionery industry rising, understanding the functionality of the bulking agents in sugar-free dark chocolate manufacture would be of significant importance in predicting changes in quality. The acquired knowledge would impact on process improvement and new product development in sugar-free dark chocolate manufacture.

Recommendations for further studies

A number of points have been noted throughout this study that suggest the need for further in-depth investigations. These could include the following:

- i. Further research is required to study tempering behaviour of the sugar-free dark chocolate with varying PSD and fat content using response surface methodology and to evaluate effects of tempering and fat crystallisation behaviour on microstructure and quality attributes (mechanical properties, appearance and melting characteristics) of the developed sugar-free dark chocolate.
- ii. Time intensity procedures could be employed to characterise the effects of optimal-temper and over-temper regimes on the melting behaviour of products during consumption. It could also be deployed to study effects of varying PSD and fat content on reported variations in melting character of derived sugar-free dark chocolates.
- iii. The effect of dry conching time and temperature on moisture content and other quality attributes of the developed sugar-free dark chocolate could also be studied. Dry conching time and temperature could be increased gradually to achieve optimum chocolate flow properties as increase in temperature would facilitate the breaking of the cohesive forces between the particles and result in effective moisture evaporation.
- iv. Milk chocolate solids comprise of particles from sugar, non-fat milk components and cocoa. This study investigated PSD of solids from only inulin, polydextrose and cocoa, which cannot be related directly to milk chocolate products. Changes in the sizes of particles from the two particulate ingredients should also be investigated for effects on the physical and sensory character in milk chocolates.

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Curriculum Vitae (Roger Philip AIDOO)

Personal Details

Email: pphilipaidoo150@hotmail.com

Nationality: Ghanaian

Contact: +32465951697

Summary of Qualifications

- **MSc. Food Technology** (2006 - 2008)

Ghent University / Katholeik University, Belgium

- **BSc. Food Science and Nutrition** (2001 – 2005)

University of Ghana, Legon

Work Experience

- **PhD Student** (2010 – 2014)

Laboratory of Food Technology and Engineering (FTE)

Ghent University / University of Ghana

- **Research Assistant** (2009 – 2010)

Alliance for Green Revolution in Africa (**AGRA**)

- **Teaching Assistant** (2005 – 2006)

Dept. of Nutrition and Food Science, University of Ghana, Legon.

Research Activities

- Functionality of inulin and polydextrose in stevia or thaumatin sweetened dark chocolate (Doctoral thesis).
- Hypochlorite - induced oxidation of whey proteins: Impact of oxidant concentration and pH (Masters thesis).
- Effect of fermentation, cowpea fortification and amylase rich flour (ARF) on the quality characteristics of nixtamalized maize and derived products. (Undergraduate project work).

Scientific Publications

- **Aidoo, R. P.**, Afoakwa, E. O. and Dewettinck, K. (2014). Rheological properties, melting behaviours and physical quality characteristics of sugar-free chocolates processed using inulin/polydextrose mixtures and sweetened with stevia and thaumatin extracts. *LWT – Food Science and Technology*, DOI 10.1016/j.lwt.2014.08.043.
- **Aidoo, R. P.**, DeClerck, N., Afoakwa, E. O. and Dewettinck, K. (2014). Optimization of processing conditions and rheological properties using Stephan Mixer as a conche in Small-scale chocolate processing. *International Journal of Food Science and Technology*, 49 : 740-746.
- **Aidoo, R. P.**, Afoakwa, E. O. and Dewettinck, K. (2014). Optimization of inulin and polydextrose mixtures as sucrose replacers during sugar-free chocolate manufacture - rheological, microstructure and physical quality characteristics. *Journal of Food Engineering*, 226 : 1259-1268.
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